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Original Research Article

Histological Evaluation of Fracture Healing after Thyroidectomy

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Abstract: This study aimed to examine the impact of thyroidectomy on the process of fracture healing. A total of sixteen adult male rabbits of the local breed were utilized. The animals were segregated into two equivalent groups, namely the control group and the treatment group. The animals were administered atropine sulfate (1mg /Kg B.W) intramuscularly as a premedication. Ten minutes later, a combination of xylazine hydrochloride (20mg /Kg B.W) and Ketamine hydrochloride (40 mg /Kg B.W) was administered intramuscularly. The control group had the induction of a mid-shaft femoral fracture, followed by fixation with intramedullary pinning. In the treated group, the identical treatment mentioned above, which involved thyroidectomy, was performed. The clinical examination showed that the inflammatory symptoms, such as swelling, discomfort, and increased temperature in the surgical site, were more severe in the control group compared to the treated group. The indications vanished during a span of four to five days in the treatment group and five to six days in the control group. The histological analysis showed that the control group had a greater presence of granulation tissues, trabecular bone, and compact bone compared to the treated group. Ultimately, it was seen that the fracture healing process was more advanced in the control group compared to the treated group.
Keywords: Thyroid gland; Fracture Healing; Thyroidectomy.

INTRODUCTION

Bones are structural structures in the body that serve various crucial roles. They provide support for soft tissues and internal organs, facilitate mobility, and help regulate calcium levels in the blood as they are the primary supply of calcium in the body (Butter, 1975; Dellman and Brown, 1976; Remedios, 1999). Fractures pose significant challenges in orthopedic surgery, as the process of fixing and repairing them involves a series of intricate physiological and biochemical processes that impact the health of both humans and animals (Ding wall, 1974; Mullis *et al*., 2021). Orthopaedic surgeons have made significant progress in the past twenty years in improving their capacity to repair and restore the form and function of injured bones and joints. By employing innovative techniques of internal fixation, external fixation, and rehabilitation, they are now able to effectively treat even the most severe fractures (Buckwalter, 2004). Unlike other mature tissues, which produce fibrous tissue at the location of an injury, the skeletal system undergoes healing by creating new bone that is identical to the surrounding, undamaged tissue. Certain elements of this process of adult regeneration share similarities with the aggregation of fetal and adult skeletal progenitor cells, which eventually form cell condensations and develop into skeletal tissue (Thompson *et al*., 2002; Luisetto *et al*., 2020). Early in the process of fracture healing, the restoration of blood vessels is a crucial step. Increasing the process of angiogenesis can enhance the development of new bone tissue (Saifzadeh *et al*., 2009). Thyroid hormones have a significant impact on the cardiovascular system, namely on the regulation of heart rate (HR). In animal models, hypothyroidism is characterized by a low heart rate, while hyperthyroidism is linked to tachycardia (Dillmann, 1996; Vargas-Uricoechea *et al*., 2014; Abdelatif and Saeed, 2016). Thyroid hormones elevate the amount of oxygen used in the peripheral tissues and the amount of substances needed, leading to a subsequent rise in the contractile strength of the heart (Sievert, 2017). Thyroid hormones also have the ability to regulate the strength and rhythm of the heart's contractions (Klein and Ojamaa, 1998). Thyroid hormones exert significant effects on the immune response of animals. Multiple investigations have demonstrated that thyroid hormones have immunomodulatory effects in animals. Administering thyroid hormones to healthy animals causes an increase in the number of white blood cells, a condition known as leukocytosis (Gupta *et al.*, 1983; Botella-Carretero *et al.*, 2005; Jara *et al.*, 2017; Galbiati *et al.*, 2021). This study aimed to assess the impact of thyroidectomy on the process of fracture healing.

MATERIALS AND METHODS

Sixteen mature male rabbits of local breed, weighting (1-1.6) Kg B.W. were used**.** They were healthy as presented by their clinical and physical examination, and housing under similar condition and feeding. The animals were divided randomly into two equal groups: First group (control): Induced mid-shaft femoral fracture, and the fracture ends fixed by intramedullary pinning. In second group (treated): Which was similar to first group but thyroidectomy was done. The animals were fasted for 24 hours for food and 12 hours for water before operation. The surgical site which included lateral and medial side of thigh in addition the neck in second group were prepared under aseptic technique. Penicillin-streptomycin at a dose of 10000, 20 mg/kg B.W intramusculary respectively was given one hour before operation to reduce the risk of postoperative infection. The animal were given atropine sulphate at a dose of 1mg/kg B.W intramusculary as a premedication 15 minutes later a mixture of xylazine hydrochloride (2%) 20 mg/kg B.W and ketamine hydrochloride (5%) 40 mg/kg B.W intra-muscularly and repeated as a half dose if needed. Animal was fixed on lateral recumbency. In control group: Longitudinal skin incision was made on lateral side of the thigh between the great trachontar of femur and the lateral aspect of the patella (Fig 1), then sharp incision of the fascia, and blunt dissection between biceps femoris and vastus lateralis muscles, to exposure of femoral bone (Fig. 2), introduce curved artery forceps beneath the bone to facilitated of the wire saw to induce transverse fracture through the mid-shaft of the bone (Fig. 3). The fracture ends were fixed by intra-madullary pinning (steinmenn stainless steel 2.4 Q x 120 mm) by retrograde technique (Fig 4, 5, and 6). The muscle and fascia was closed by simple continous pattern, using cat gut size (2.0). Skin was sutured with simple interrupted pattern by silk size (2.0). While in treated group using same the procedure as describe in control group except that the thyroidectomy was done after fixation of two fracture ends. Penicillin –streptomycin in a dose of 10000 I.U, 20 Mg /Kg B.W intramuscular respectively for four days post-operation, and all the animals were kept under observation during the exponents period and skin suture was removed ten days after operation.

Figure 1: Photograph shows longitudinal incision in lateral aspect of thigh.

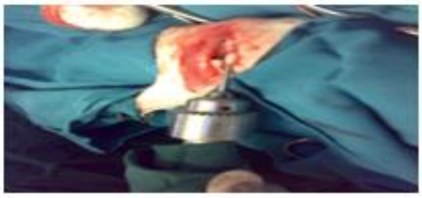

Figure 2: Photograph shows exposure of femoral bone.

Figure 3: Photograph shows introduce of wire saw beneath the mid - shaft of femoral bone.

Figure 4: Photograph shows introduce the of steinman pin in bone morrow of proximal end of femur by (retrograde method).

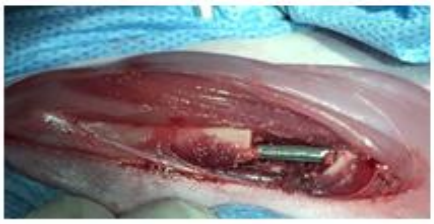

Figure 5: Photograph shows introduce of pin in distal segment of fracture end

Figure 6: Photograph shows applied a ligament between two bone fragments

RESULTS AND DISCUSSION

The clinical examination findings indicated that the control group had inflammatory indications starting from the second day after the operation. These signs included swelling at the site of the operation, elevated temperature in the region, discomfort, and inability to bear weight on the afflicted legs. Within a period of five to six days following the procedure, the swelling had completely subsided, and the animal was able to bear weight on the injured limb. The sensation of movement in the fracture ends was there initially, but it ceased at the start of the third week.

In the treated group, all of the aforementioned symptoms were less pronounced compared to the control group. Furthermore, these symptoms completely resolved by five days after the surgery. The movement of the fractured ends ceased by the end of the fourth week. The presence of edema, redness, pain, and increased heat at the surgical site on the second day after the operation may be attributed to increased blood flow in the area, as well as the dilation and increased permeability of blood vessels, resulting in the migration of white blood cells and inflammatory cells outside of the blood vessels and the subsequent formation of edema at the surgical site. This observation was agreed with other workers (Jones and Hunt, 1983).

The discomfort may be attributed to the edema surrounding the fractured location, resulting in heightened pressure on the nerve endings. The degree of inflammation stimulates cells to produce prostaglandin, which in turn leads to vasodilation and increased infiltration of blood vessels, resulting in the buildup of exudate in the fracture region. The signs disappeared within four to five days after the operation in the control group. However, in the treated group, the clinical signs were less severe compared to the control group. This could be attributed to the vascular effect of thyroid hormones, which stimulate the production of many phagocytes cells at the surgical site. This increased the severity of tissue inflammation. Thyroid hormones have a highly vascular effect and are known to promote the growth of blood vessels in nearby tissues. This finding is consistent with the research conducted by Parfitt in 1976.

Furthermore, the tearing of the blood supply to the bone and surrounding tissues leads to the exudation of plasma and white blood cells. This results in a decrease in oxygen levels and an increase in acidity in the affected area, leading to swelling and inflammation. As a result, necrotic tissues are removed (Delves *et al*., 2017; Leung, 2021).

Table 1: Average thyroxin hormone concentrations pre- and post-Thyroidectomy

Groups	Pre- Thyroidectomy	Post-Thyroidectomy
Control	56.23± 14.21 nmol/L [a]	49.63± 45.27 nmol/L[a]
Treated	52.71± 18.01 nmol/L[a]	0.23± 21.11 nmol/L[b]

a, b within each row, averages marked with different letters differ considerably in terms of significance ($p \leq 0.05$).

Hormonal Assay in control group shows, there is no significant difference between its values at pre-thyroidectomy (56.23± 14.21 nmol/L) when compared with Post-thyroidectomy (49.63± 45.27 nmol/L) (Table 1). The results of the clinical and radiographic examination showed that the fracture healing was not affected, and it was within the normal rates, and this is due to the stability of the factors affecting the healing, the most important of which is the hormone thyroxin. shows transformation of most of the cartilage tissue to bone tissue, characterized by an increase in the thickness and regularity of bone trabeculi, as well as proliferation of osteoblasts that lining the bone morrow. This is consistent with what he found (Chmurska *et al.*, 2021).

The hormonal assay in the treatment group demonstrates a substantial change in results between the pre-thyroidectomy (52.71± 18.01 nmol/L) and post-thyroidectomy (0.23± 21.11 nmol/L) periods (Table 1). The process of bone fracture healing was shown to be considerably impacted, with instances of delayed healing and non-healing seen. This conclusion was supported by the findings of both clinical and radiographic examinations. The primary cause for the delay in healing may be the cessation of the thyroxine hormone's influence since it plays a crucial part in facilitating the process of fracture healing. This aligns with the findings you have obtained (Lo *et al.*, 2002; Reddy *et al*., 2016).

At 2 weeks after the procedure, histopathological analysis of the control group revealed clogged blood vessels with an increase in fibroblast cells that invaded the fracture site and generated connective tissue fibers (Fig. 7). Trabecular bone growth is shown, along with the presence of connective tissue fibers. Additionally, there is infiltration of macrophages and plasma cells. Furthermore, there is evidence of osteoblast proliferation, resulting in the production of osteoid tissue as shown in Figure 8 and 9. At 4 weeks after surgery, the examination reveals the presence of dense bone tissue with Haversian canals (Fig 10), proliferation of osteoblasts, with increase in the thickness and regularity of bone trabeculi ‘(Fig 11).

At 2 weeks after surgery in the treated group, there is an organized hematoma between the two fracture fragments. This hematoma is characterized by the presence of a network of connective tissue fibers and infiltration with inflammatory cells such as neutrophils, macrophages, and fibroblasts (Fig 12). Additionally, there is the formation of fibrous tissue with the presence of necrotic osteocytes that are devoid of nuclei in the lacunae (Fig 13).

This is a consequence of reduced levels of thyroid hormones, which is consistent with the findings of Kalfas, 2001 and Loi *et al*., 2016.

During the progression of vascular ingrowth, a collagen matrix is deposited as osteoid is produced and then calcified. This process results in the development of a soft callus surrounding the site of repair. During the initial 4 to 6 weeks of the healing phase, this callus exhibits a low level of resistance to movement and necessitates sufficient protection through the use of bracing or internal fixation. Arazi and Canbora conducted a study in 2016. The callus undergoes ossification, resulting in the formation of a woven bone bridge between the fragments of the fracture. If adequate immobilization is not employed, ossification of the callus may fail to take place, resulting in the formation of an unstable fibrous union instead, Noviana *et al*., (2011) and Hyytiainen (2016).

The control group exhibited greater maturity in the remodeling stage compared to the treated group. This is because when the fracture site is subjected to an axial loading force, bone deposition occurs where it is required, while resorption takes place in areas where it is not needed. This finding aligns with the conclusions of Kalfas (2001), Mistry and Mikos (2005), Liu *et al*., (2017), Eliaz and Metoki (2017), and Barnsley *et al*., (2021).

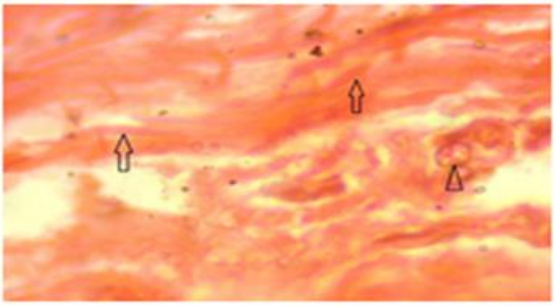

Figure (7) photomicrograph of control group at 15th day postoperation show presence of congested blood vessels (⇨) around the fracture site with proliferation of fibroblast (△) (H&E 100X).

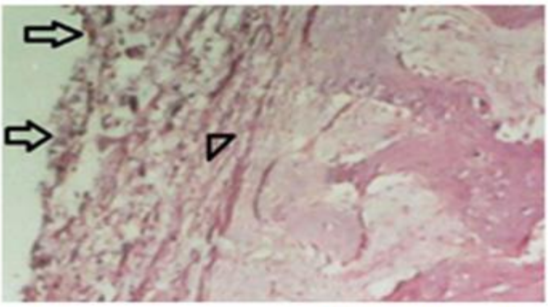

Figure (8) Control group at 15th day postoperation show external callus formation (⇨) that have little connective tissue fibers with infiltration with macrophages and plasma cells (△) (H&E 10X).

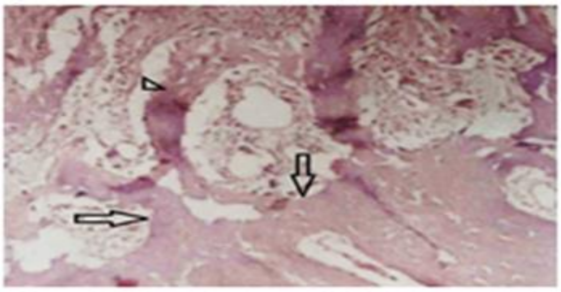

Figure (9) Control group at 15th day postoperation show narrow gaps between bone trabeculi, and proliferation of osteoblast (△)that formed osteoid tissue (⇨)(H&E 10X).

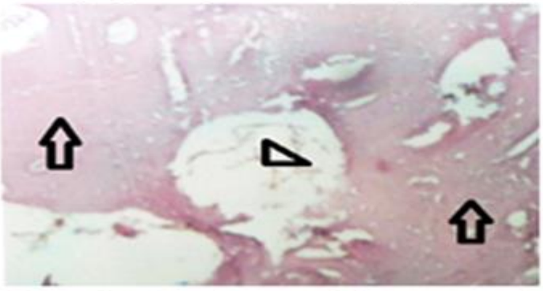

Figure (10) Control group at 30th day postoperation show the lamellar bone formation (△)instead of compact bone with increased in Haversian canal (⇨)(H&E 10X).

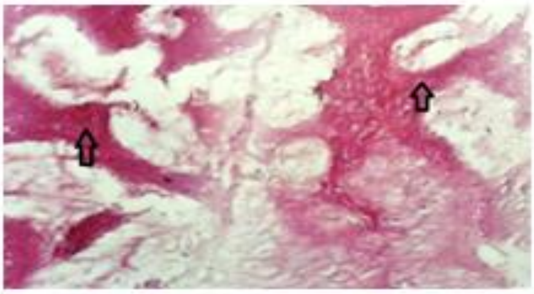

Figure (11) Control group at 30th day postoperation show internal callus marked by more thickness and regularity of bone plate and increase in the thickness and regularity of bone trabeculi (H&E 40X) ⇨

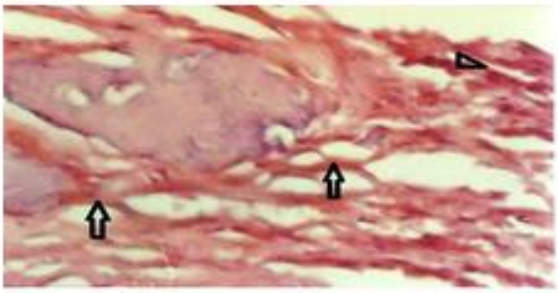

Figure (12) Treated group at 15th day postoperation show presence of a network of fibrous tissue (⇨) and infiltration of inflammatory cells (Δ) (H&E 100X).

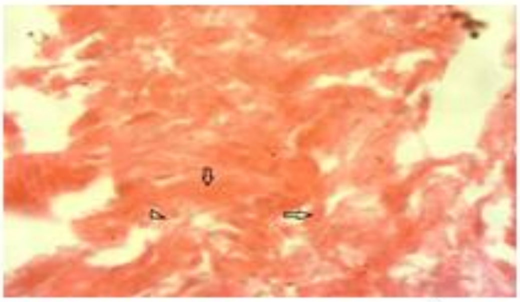

Figure (13) Control group at 15th day postoperation show fibrous tissue with presence the necrotic osteocyte that free of the nuclei in lacunae (H&E 100X).

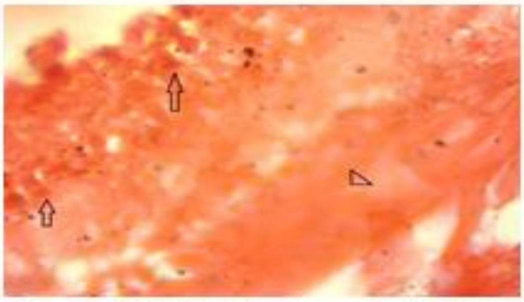

Figure (14) Treated group at 30th day postoperation show external callus formation, that marked by the transformation of most of the cartilage tissue to bone tissue(⇨)

REFERENCES

- Abdelatif, A. M., & Saeed, I. H. (2016). Effect of altered thyroid status in the domestic rabbit (Lepus cuniculus) on thermoregulation, heart rate and immune responses, *16*(4), 26-30.
- Arazi, M., & Canbora, M. K. (2016). Fracture Healing. In *Musculoskeletal Research and Basic Science* (pp. 323-341). Springer International Publishing.
- Barnsley, J., Buckland, G., Chan, P. E., Ong, A., Ramos, A. S., Baxter, M., & Patel, H. P. (2021). Pathophysiology and treatment of osteoporosis: challenges for clinical practice in older people. *Aging Clinical and Experimental Research*, *5*(7), 1-15.
- Bader, O. A., & Nahi, H. H. (2023). Effect of estrogens level on the fracture healing of tibia bone after ovariectomy and ovariohysterectomy in female dogs. *Iraqi Journal of Veterinary Sciences*, *37*(4), 921-928.
- Botella-Carretero, J. I., Prados, A., Manzano, L., Montero, M. T., Escribano, L., Sancho, J., & Escobar-Morreale, H. F. (2005). The effects of thyroid hormones on circulating markers of cell-mediatedimmune response, as studied in patients with differentiated thyroid carcinoma before and duringthyroxine withdrawal. *European Journal of Endocrinology*, *153*(2), 223-230.
- Buckwalter, J. A. (2004). Can tissue engineering help orthopaedic patients? Clinical needs and criteria for success. In: Sandell, L. J., Grodzinsky, A. J., eds. Tissue engineering in musculoskeletal clinical practice. Rosemont, IL: American Academy of Orthopaedic Surgeons, 3–16.
- Butter, H. C. (1975). Resume of fracture healing. In: The veterinary clinics of (north America, *5*(2), 147-157.
- Dellman, H. D., & Brown, E. M. (1976). Connective arc supoortve tissue in textbook of veterinary histology. INC. Lea and febiger ¬philadelphia.74-75.
- Delves, P. J., Martin, S. J., Burton, D. R., & Roitt, I. M. (2017). *Essential immunology*. John Wiley & Sons.13th Ed Pp:130-134.
- Eliaz, N., & Metoki, N. (2017). Calcium Phosphate Bioceramics: A Review of Their History, Structure, Properties, Coating Technologies and Biomedical Applications. *Materials*, *10*(4), 334.
- Milosevic, V. (2017). Response of trabecular bone, thyroid C and follicular cells to synthetic salmon calcitonin in middle-aged orchidectomized male rats. *Journal of Anatomy*.
- Galbiati, V., Buoso, E., d'Emmanuele di Villa Bianca, R., Paola, R. D., Morroni, F., Nocentini, G., & Corsini, E. (2021). Immune and Nervous Systems Interaction in Endocrine Disruptors Toxicity: The Case of Atrazine. *Frontiers in Toxicology*, *4*(3).
- Gupta, M. K., Theresa, C., & Deodhar, S. D. (1983). Effect of thyroxine on immune response in C57B1/6Jmice. *Acta Endocrinologica (Copenh)*, *103*(1), 76-80.

- Hyytiainen, H. (2016). Small animal treatment and rehabilitation for neurological conditions. *Animal Physiotherapy: Assessment, Treatment and Rehabilitation of Animals*, 260.
- Jara, E. L., Munoz-Durango, N., Llanos, C., Fardella, C., Gonzalez, P. A., Bueno, S. M., & Riedel, C. A. (2017). Modulating the function of the immune system by thyroid hormones and thyrotropin. *Immunology Letters*.
- Kalfas, I. H. (2001). Principles of bone healing. *Neurosurgical focus*, *10*(4), 1-4.
- Klein, I., & Ojamaa, K. (1998). Thyrotoxicosis and theheart. *Endocrinology and Metabolism Clinics of North America*, *27*(1), 51-62.
- Liu, W. C., Chen, S., Zheng, L., & Qin, L. (2017). Angiogenesis Assays for the Evaluation of Angiogenic Properties of Orthopaedic Biomaterials–A General Review. *Advanced Healthcare Materials*.
- Loi, F., Cordova, L. A., Pajarinen, J., Lin, T. H., Yao, Z., & Goodman, S. B. (2016). Inflammation, fracture and bone repair. *Bone*, *5*(86), 119-130.
- Leung, E. K. Y. (2021). Parathyroid hormone. In *Advances in Clinical Chemistry*, *101*(23), 41-93.
- Mistry, A. S., & Mikos, A. G. (2005). Tissue engineering strategies for bone regeneration. In *Regenerative medicine II* (pp. 1-22). Springer Berlin Heidelberg.
- Mullis, B. H., Gudeman, A. S., Borrelli Jr, J., Crist, B. D., Lee, M. A., & Evans, A. R. (2021). Bone healing: Advances in biology and technology. *OTA International*, *4*(2S), 100.
- Noviana, D., Soedjono, G., Abdullah, D., Soehartono, R. H., Ulum, M. F., Siswandi, R., & Dahlan, K. A. (2011). In vivo study of hydroxyapatite-chitosan and hydroxyapatite-tricalcium phosphate bone graft in sheep's bone as animal model. In Instrumentation, Communications, Information Technology, and Biomedical Engineering (ICICI-BME), 2011 2nd International Conference on (pp. 403-408). IEEE.
- Remedios, A. (1999). Bone and bone healing. In Fracture management bone healing. Edit by Martine, S.A. 29 (5): 1029-1044.
- Root, A. W. (2021). Disorders of Mineral Metabolism: Normal Homeostasis. In Sperling *Pediatric Endocrinology,* (123), 220-278.
- Saifzadeh, S., Pourreza, B., Hobbenaghi, R., Naghadeh, B. D., & Kazemi, S. (2009). Autogenous greater omentum, as a free non vascularized graft, enhances bone healing: an experimental non-union model. *Journal of investigative surgery*, 22, 129-137.
- Sievert, L. L. (2017). Thyroid Disorders at Midlife: An Evolutionary Perspective. In *The Arc of Life*, *12*(6), 121-138.
- Smit, M. A., van Kinschot, C. M., van der Linden, J., van Noord, C., & Kos, S. (2019). Clinical guidelines and PTH measurement: does assay generation matter?. *Endocrine reviews*, *40*(6), 1468-1480.
- Thompson, Z., Miclau, T., Hu, D., & Helms, J. A. (2002). A model for intramembranous ossification during fracture healing. *J Orthop Res*, 20, 1091-1098.
- Vargas-Uricoechea, H., Bonelo-Perdomo, A., & Sierra-Torres, C. H. (2014). Effects of thyroid hormones on the heart. *Clínica e Investigación en Arteriosclerosis*, *26*(6), 296-309.
- Chmurska-Gąsowska, M., Sowińska, N., Pałka, S., Kmiecik, M., Lenarczyk-Knapik, J., & Migdał, Ł. (2021). Non-Invasive Measurement of Thyroid Hormones in Domestic Rabbits. *Animals*, *11*(5), 1194. https://www.mdpi.com/2076-2615/11/5/1194
- Lo, C. Y., Luk, J. M., & Tam, S. C. (2002). Applicability of intraoperative parathyroid hormone assay during thyroidectomy. *Annals of surgery*, *236*(5), 564. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1422613/
- Reddy, A. C., Chand, G., Sabaretnam, M., Mishra, A., Agarwal, G., Agarwal, A., & Mishra, S. K. (2016). Prospective evaluation of intra-operative quick parathyroid hormone assay as an early predictor of post thyroidectomy hypocalcaemia. *International Journal of Surgery*, 34, 103-108. https://www.sciencedirect.com/science/article/pii/S1743919116302990 ===]