Autotransplantation of thyroid tissue in rats. An experimental study

Ann. Ital. Chir., 2008; 79: 389-396

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BACKGROUND: *The aim of this study is to demonstrate the functional capacity of thyroid autografts after total thyroidectomy in a rat model.*
MATERIALS AND METHODS: *60 rats were divided into 6 groups of 10 rats each. Thyroid gland was cut into 0.5 mm pieces and was inserted intramuscularly into the left rectus abdominis muscle at different time intervals following total thyroidectomy. The animals were observed for 30 days. Blood samples were collected weekly for TSH, FT3 and FT4 measurements. This study was conducted in strict accordance with the provisions of the law concerning test procedures on animals, as per Legislative Decree n.116/92.*
RESULTS: *Autologous transplantations were successful in 70% of the cases. Histopathological findings showed normal thyroid architecture.*
It was observed that thyroid function recovered was more rapidly if the implants were performed immediately after thyroidectomy than in implants performed at a later time.
These results could be due to the thyroid tissue preserving procedure used which may have led to reduce the restored thyroid function in the groups of animals where the implantation was not immediate.
CONCLUSIONS: *Ectopically transplanted thyroid tissue is able to survive and recover its function completely if maintained vital in an adequate preserving medium.*

KEY WORDS: Autotransplantation, Rat, Thyroid, Total thyroidectomy.

Introduction

Patients undergoing total thyroidectomy are frequently managed postoperatively with exogenous thyroxine or other thyroid supplementation to suppress the resulting increased TSH levels in the serum. Although thyroid hormone treatment can be effective in compensating for organ loss, patients continue to suffer from subclinical depression, weight gain. Administration of thyroid hormone is a common therapy for hypothyroidism which is thought to be associated with low stress and only slight inconvenience for the host. However, this therapy involves daily administration of levothyroxine at a dose which must be continually adjusted to the blood thyroxine level throughout the rest of the patient's life.
Moreover, long-term thyrotropin-suppressive therapy with levothyroxine impairs small and large artery elasticity, increases left ventricular mass[1] and modifies the lipid profile[2].
The principle of immediate or delayed autotransplantation of the endocrine gland or part of it, after total or subtotal resection, in order to avoid postoperative functional insufficiency, was first proposed by Halsted in 1909[3]. Literature reports remarkably positive results in case of autografts of parathyroids[4], isles of Langherans[5], adrenal cortex[6], testicle and ovary[7]. Thyroid autotransplantation in animal models has been previously reported by other Authors for example Papaziogas et al.[8] for

Pervenuto in Redazione Gennaio 2008. Accettato per la pubblicazione Luglio 2008.
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rabbits, Popov et al.[9] for rats, and Gàl et al.[10] for dogs. According to literature data, autotransplantation of thyroid tissue for postoperative hypothyroidism remain controversial in clinical practice.
The aim of this study is to examine the possibility of autotransplantation of thyroid tissue in the rectus abdominis muscle, and to evaluate thyroid function postoperatively in rats. We hypothesized that reimplantation of functioning thyroid tissue could cure patients with acquired hypothyroidism by reducing their need for exogenous thyroxine.

Materials and Methods

60 Wistar Hannover rats, Harlan breed, 30 males and 30 females, with a mean age of 3 months (range: 2 to 4 months) and a mean weight of 300 grams (range: 210 to 315 grams) were included. All animals were maintained under the same condition and received the same quantities of water and food. Rats were divided into 6 groups (from A to F) of 10 animals each, which were uniform in sex, weight and age. All animals underwent serial serum TSH, FT3 and FT4 samples using the technique of collecting blood samples from the retro-orbital venous plexus under ether anaesthesia.
60 rats underwent total thyroidectomy. The thyroid gland was cut into 0.5 x 0.5 mm pieces and was inserted intramuscularly into the muscular pouch of the left rectus abdominis muscle.
Group A (control group) consisted of 10 healthy rats who underwent only weekly blood samples to determine the normal values of thyroid functional indexes in this kind of rats.
Another group of 10 rats (group B, reference group) underwent total thyroidectomy, without autologous implantation, and serum samples 24 hours before surgery (time 0) and subsequently on 7th, 14th, 21st and 30th postoperative day.
After total thyroidectomies in the other 40 rats from groups C, D, E and F, autografts of fragmented thyroid tissue were placed in a muscular pouch at different times: immediate autograft in group C, autograft after 24 hours after surgery in group D, after 7 days in group E, and after 14 days in group F. In these groups, blood samples were collected 24 hours before surgery (time 0) and on 7th, 14 th, 21 st and 30 th day after the autograft. On the 30th day all animals were sacrificed, and the muscular pouch, site of the autograft, was removed for histopathological examination.
All blood samples were centrifuged (5000 rpm, 20 min). Serum samples were kept in paraffin-sealed test tubes and stored at -22°C. Thyroid hormones were measured by radio-immuno assay (RIA) kits (Immunotech® Company, 130 Avenue de Tassigny, Marseille, France) at the hormone laboratory of Department of Nuclear Medicine of the "Maggiore" Hospital in Trieste. The normal values indicated were: FT3 (2.43±0.78 pg/mL), FT4 (4.69±1.85 ng/dL), TSH (1.77±0.40 µIU/mL).
Statistical analysis of the results was performed using SPSS software (Windows), and the analysis of variance with repeated measures for TSH, FT3 and FT4 levels evaluation. A p value < 0.05 was considered statistically significant.
Thyroidectomies were performed under total anaesthesia induced by intra-peritoneal injection of 2.4 cc of Avertina and 0.1 cc of Xilazina at 2.0%.
Survival and function of the grafts were evaluated by: restoration of normal levels of serum thyroxine, weight gain, and histological findings.
This study was conducted in strict accordance with the provisions of the law concerning test procedures on animals, as per Legislative Decree n.116/92.

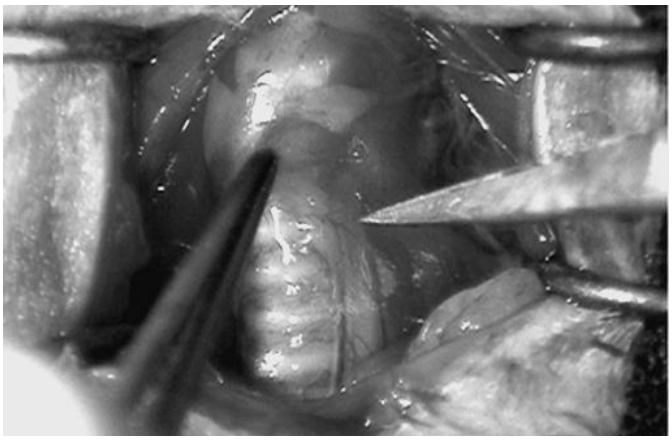

Fig. 1: Left recurrent laryngeal nerve, the two superior and inferior thyroid arteries, and the anterolateral side of the esophagus (x 10).

Total thyroidectomy
A midline longitudinal cervicotomy of about 2-3 cm length was performed. The major sublingual and submaxillary glands were placed laterally. The tracheal plane was reached by displacing the muscular fibres of the sternohyoideus muscles and the sternothyreoideus muscles, both lying superficially and centrally, and of the omohyoideus muscles, also placed in a central position but a little deeper than the above-mentioned muscles.
At the tracheal plane, it was possible to identify the two thyroid lobes, the isthmus and the two upper parathyroid glands. The thyroid capsule was excised from the adjacent structures. The superior and inferior thyroid arteries were identified and ligated with transfixed stitches 8/0. During the dissection of the two thyroid lobes from surrounding tissues, the upper parathyroid glands were identified and preserved. Finally, the thyroid was removed from the trachea, haemostasis was checked and the skin was closed with interrupted sutures of silk 4/0 (Fig. 1).

Preparation of the thyroid autograft for implantation
The removed thyroid was immersed in a sterile Petri's

capsule containing Hank's balanced solution and was cut into 0.5 x 0.5 mm pieces. The fragments, until implantation, were placed into an adequate sterile container with Hank's balanced solution and stored in the fridge at +4°C[11].

Autologous transplantations of thyroid tissue fragments were carried out under ether anaesthesia since the duration of the surgery is short. A 2 cm longitudinal incision was performed at the level of the left medial-inferior quadrant of the abdomen. The superficial fascia of the left rectus abdominis muscle was dissected and its fibres were divaricated. Thyroid fragments were placed intramuscularly at a certain distance one from the other, so as to allow for wider contact surface between the thyroid and the muscular tissues. Then, the muscular pouch was closed accurately with non-absorbable 8-0 stitches, so as to allow the site of the graft to be detected in order to remove the muscular pouch when the rat is sacrificed.

Method of the collection of blood samples from the retro-orbital plexus

The retro-orbital plexus is a commonly used site for periodic sampling during the course of a study. This method has been shown to be reliable for the repeated collection of blood samples.

Blood is obtained from the retro-orbital plexus in the anesthetized rat.

The rat is placed in lateral or ventral recumbency. A Pasteur pipette or microhematocrit capillary tube is passed beneath the upper lid at the medial canthus (inside corner) of the eye. The tube is gently pushed and twisted until it penetrates the conjunctiva. Without removing the tube from beneath the lid, the tube is gently retracted until blood flows into the tube. Once the flow is started, tilting a Pasteur pipette can allow gravity to assist. When the sample was obtained, the tube is removed from the eye, and the lids are immediately held closed with a dry gauze sponge for several seconds, to achieve hemostasis. This technique is used to obtain amounts of blood from 5 to 3 mL. No corneal or ocular damages with this technique in our experience were observed.

Results

After 4 initial deaths at the outset mostly due to too deep ether anaesthesia, no other negative results that can be ascribed to the surgical procedures used in this experimental study occurred.

A group of 10 rats was used in the study for testing a surgical procedure and a blood sampling technique. Within these animals 4 rats died initially due to too deep anaesthesia. This group of animals was excluded from the statistical analysis and was not enrolled in the study.

No negative results can be ascribed to the surgical procedures. Only the animals with functioning grafts were included in the statistical analysis.

No differences were found in terms of gender between different groups.

The results obtained for each group showed that in group A (control group), serum TSH, FT3 and FT4 levels were uniform, in concordance with the normal ranges set by the Company that supplied the kits for RIA hormone dosage (Figs. 2-4).

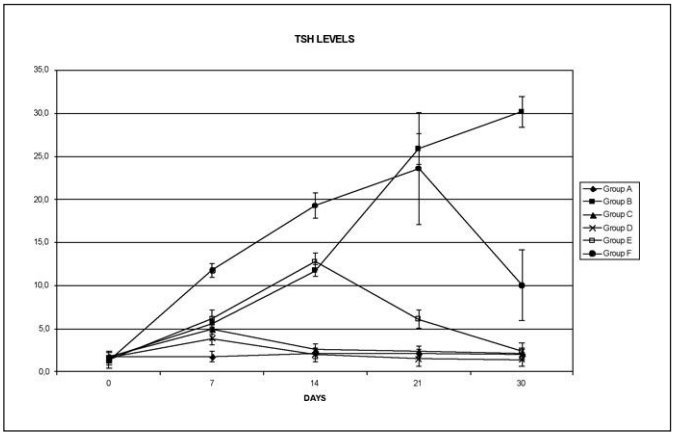

Fig. 2: Levels of TSH in all groups.

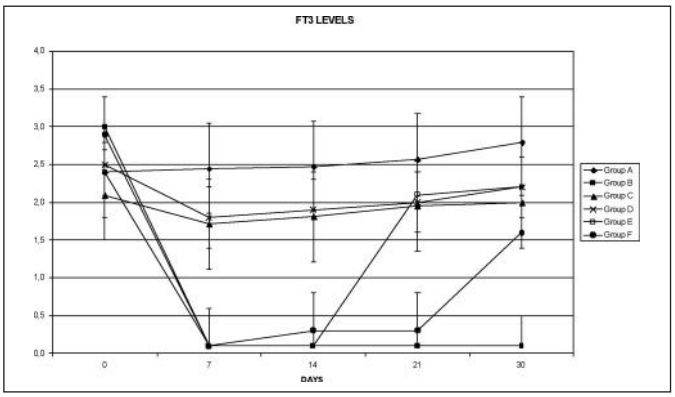

Fig. 3: Levels of FT3 in all groups.

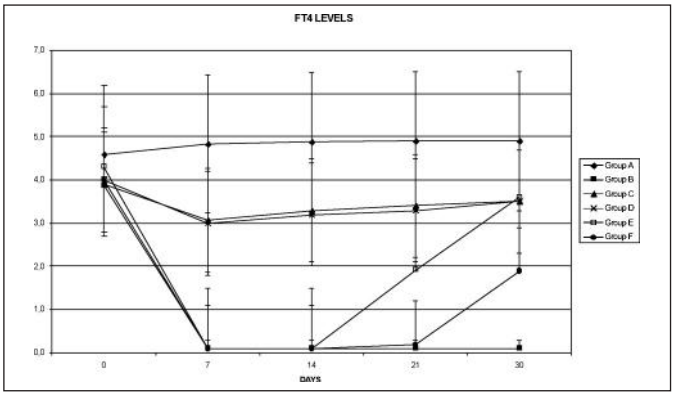

Fig. 4: Levels of FT4 in all groups.

After total thyroidectomy all rats of group B (reference group) rapidly developed a condition of total athyroidism that continued until the end of the observation period. Already at 7 days from surgery it was possible to observe the undetectable levels of thyroid hormones and a rapid and remarkable increase in TSH levels (Figg. 2-4).

In group C rats (immediate engraftment) the trend of TSH, FT3 and FT4 levels shows how the graft survived and recovered its functions almost immediately in 8 rats out of 10 (80.0%). The remaining two rats showed a condition of athyroidism for the whole period of observation (Figs. 1-3).

In group D (engraftment after 24 hours) 7 rats out of 10 (70.0%) showed hormone levels that, after a slight decrease after 7 days from the engraftment, returned to almost normal levels on the 14th day and which were maintained until the 30th day of observation. However in 3 rats, thyroidectomy induced athyroidism which not modified by the engraftment that, did not survive in these animals (Figs. 1-3).

In group E (engraftment after 7 days), at the moment of transplantation in all rats, FT3 and FT4 levels were undetectable and TSH levels were remarkably higher than normal levels, demonstrating a total athyroidism. At the end of the observation period, 7 rats out of 10 (70.0%) showed a good recovery of thyroid function that, nevertheless, was in all rats delayed compared to the thyroid function recovery observed in groups C and D in which engraftment was performed rapidly (Figg. 2-4).

In group F (engraftment after 14 days) only one rat showed detectable levels of FT3 and FT4 at 14 days after transplantation. Thereafter, these levels increased at a constant rate. After 30 days from the engraftment, in other 5 rats, thyroid hormone levels were detectable, showing levels near to the lowest normal limits. Therefore, the graft in this group survived in 60.0% of the animals (Figs. 2-4).

Figure 1 shows the levels of TSH in all groups. While all groups demonstrate similar values at time point 0, group F differs significantly at day 7 and day 14 from all other groups. A possible explanation of the differences between group F and group B and E at these time points might be from the assay used, because the kit was replaced.

To sum up, no intra-operative mortality occurred. No statistically significant difference ($P<0.05$) was observed among the various groups at the beginning of the study. On the 7th day after the engraftment, groups C and D showed FT3 and FT4 levels remarkably higher, though not yet normal compared to those of the control group, than those of groups B, E and F.

TSH levels resulted extremely heterogeneous: this datum can be ascribed to a intrinsic bias in the protocol of the study as a consequence of which the engrafted groups to remain, for a variable periods, in athyroidism (from a few hours to 14 days) during which TSH hyper-incretion develops so as to make it impossible to compare the hormone levels after a short post-operative period. After 14 days the hormonal situation resulted substantially the same as before surgery for FT3 and FT4 levels. The groups engrafted early (groups C and D) showed a stabilization of TSH levels compared to those of control group A. In the delayed engrafted groups (groups E, F), TSH levels resulted significantly ($P<0.05$) higher than those of the other groups. In group F, which was kept under a condition of athyroidism for a period of 14 days, it was also possible to observe that TSH levels were remarkably higher than those of group E (7 days of athyroidism) and that both the groups showed significantly higher values than those of group B, thus confirming the above-mentioned datum.

Groups C and D showed, at the end of the 3 rd week, hormone profiles as same as the control group. However, group E showed thyroid function recovery with FT3 levels comparable to those of the control group and FT4 levels remarkably higher than those of groups B and F, yet still lower than those of group A. Moreover, in group E it is also possible to observe a decrease in TSH incretion which tends to normality.

At the 30th post-operative day, it was possible to observe a recovery in the gland function of group F: FT3 and FT4 values, though not yet falling into normal ranges compared to those of the other groups, were significantly higher than those of group B and also TSH tended to normal levels as had occurred in group E on the 21st postoperative day.

Histological examination of the 28 successfully engrafted rats, showed, in all preparations examined, the apparent presence of normo-functional thyroid tissue characterised by functional and mature thyrocites, thyroid follicles full of colloid and by a newly developed peripheral vascularization (Fig. 5).

The remaining engraftments (12/40), showed a presence of necrotic areas surrounded by granulation tissue with giant cells due to granuloma caused by an extraneous body (Fig. 6).

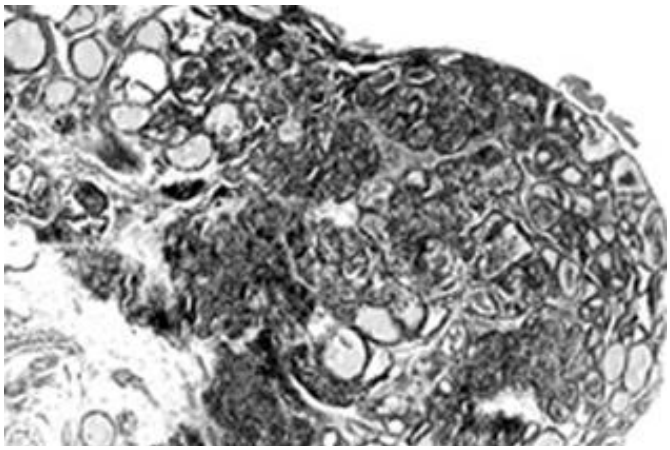

Fig. 5: Thyroid tissue in the muscular pouch at the moment of the sacrifice (x 400).

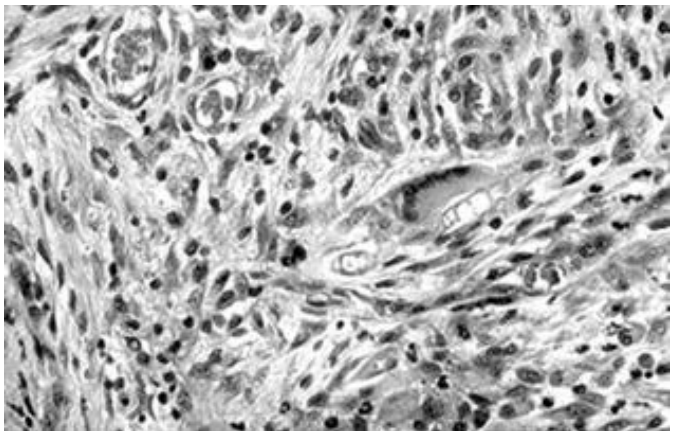

Fig. 6: Necrotic areas surrounded by granulation tissue with giant cells, a typical picture of granuloma due to an external body (x 400).

Our data show that, in the transplanted rats, it is possible to observe a delayed recovery of thyroid function in relation to the interval between total thyroidectomy and engraftment. This datum shows the wide standard deviations observed in the groups of successfully engrafted rats.

Therefore, the trend of the hormone profiles of each group of rats was examined on the basis of the time elapsed between thyroidectomy and transplantation.

It is possible to observe that in early transplanted groups (groups C,D) hormone levels are practically the same as the control group, while in delayed transplanted groups (groups E,F), after a period of 7-14 days in which TSH tends to increase and thyroid hormones are undetectable, it is possible to observe a gradual stabilization tending to normality that, however, is only partial in some cases (Tab. I).

Discussion

This study demonstrates the feasibility of total thyroidectomy in rats and that thyroid tissue grafted ectopically can survive and maintains function. The effectiveness of the surgical technique used to perform total thyroidectomy in rats is demonstrated by the results obtained in group B: the trends of TSH, FT3, and FT4 levels confirm that the removal of the thyroid was total. Indeed, all thyroidectomized rats showed a progressive and rapid hypothyroidism that eventually resulted in total athyroidism, which lasted for the whole period of the observation.

Regarding the effectiveness of ectopic autologous thyroid tissue engrafts as a therapy to treat absolute athyroidism induced by total thyroidectomy, the results obtained demonstrate that the procedure is efficacious if, the endocrine tissue to be engrafted in rats, is vital and functioning. Indeed, serial TSH, FT3 and FT4 measurements show that in all the successfully grafted animals (70.0%) thyroid function recovered after different time periods of hypothyroidism (group C) or of athyroidism (groups D, E and F). A further confirmation results from the histological examination of the muscular pouches removed, from the successfully grafted rats when sacrificed: in all cases the presence of apparently normal thyroid tissue was observed.

The fact that the number of positive results and thyroid function restoration decreases as the time elapsed between thyroidectomy and autografts increases is to be ascribed to the thyroid tissue preserving procedure used. The use of Hank's balanced solution at +4°C to preserve the removed thyroid awaiting transplantation does not seem to be fit to maintain the vitality of thyroid tissue to be grafted, as already stated by other Authors[12-14].

The thyroid tissue preserving procedure used in this study may considerably influence the restoration of the thyroid function in the groups of animals where the time elapsed between thyroidectomy and autotransplantation. Several thyroid tissue preserving procedures have been reported in literature: cryopreservation at - 80 degrees C°[15] and at - 196 degrees C° [10,16,17] with good results. However, in our study, the thyroid tissue was stored in Hank's balanced solution at +4 C° because the cryopreservation under high freezing conditions was not available in our Department, only Hank's method was available on our budget.

In the last few years, experimental studies carried out on cells having particular characteristics, stem cells, have led to the formulation of new and surprising therapeutical hypotheses that also involve the field of endocrinology[18,19]. Stem cells have been recently detected in different tissues in adults[20], isolated and cultured in vitro. Stem cells have been isolated also from the haematopoietic tissue[21], from the nervous tissue[22], from the mesenchyme[23], from the epidermis[24], from the cornea[25] and from the endothelium[26]. All these cells are able to generate lines of in vitro culture that remain stable in time, to maintain their biological features and to differentiate into mature cells of the tissue into which they have been inoculated. These interesting findings suggested starting this experimental study on totally thyroidectomized rats in order to find a specific substitute cell therapy to treat postoperative athyroidism. Therefore, the therapy does

Table I – Thyroid function restoration in all groups.

Group	Time of autograft	Success	Time of first increasing hormone levels
Group A (control group)	0	0	0
Group B	0	0	0
Group C	immediate	8/10	14 days
Group D	24 h	7/10	14 days
Group E	7 days	6/10	21 days
Group F	14 days	6/10	21 days

not consist in an autograft of mature thyroid tissue, but in a graft of new tissue, deriving from in vitro culture of adult stem cells obtained from the removed thyroid and, thus, able to develop mature thyroid tissue. This study represents only a first step of this experimental protocol and our initial experience suggest a larger study is needed to more fully examine these findings.

Conclusions

The results obtained confirmed that autografts of thyroid tissue performed in a muscular pouch are successful and able to restore normal endocrine function if maintained vital in an adequate preserving medium.
Our method presented here, may be developed as a viable strategy for the treatment of patients with acquired hypothyroidism. The observation period in our experimental study was relatively short, but the presence of vital thyroid follicles along with the restoration of normal hormone levels could be evidence of long-term efficacy of the thyroid autograft.

Riassunto

OBIETTIVO: Lo scopo dello studio è dimostrare la capacità del tessuto tiroideo del ratto di attecchire e riprendere le sue funzioni se reimpiantato in una sede ectopica.
MATERIALI E METODI: Sono stati impiegati 60 animali suddivisi in 6 gruppi di 10 ratti ciascuno. La ghiandola tiroidea asportata è stata suddivisa in frammenti delle dimensioni di circa 0.5 mm e quindi reimpiantata in una tasca muscolare a livello del muscolo retto sinistro dell'addome a intervalli di tempo diversi dall'intervento di tiroidectomia totale. Tutti gli animali sono stati osservati per un periodo di 30 giorni e sono stati sottoposti a prelievi ematici seriati settimanali per il dosaggio del TSH, dell'FT3 e dell'FT4. Questo studio è stato condotto seguendo rigorosamente le norme previste per gli studi su animali contenute nel Decreto Legislativo numero116/92.
RISULTATI: Il reimpianto autologo ha avuto successo nel 70% dei casi. L'esame istologico della tasca muscolare prelevata al momento del sacrificio dimostrava la presenza di tessuto tiroideo con architettura conservata apparentemente normofunzionante.
Si è osservato che tanto più precocemente viene effettuato l'autotrapianto, tanto maggiore è la capacità di attecchire del tessuto tiroideo e tanto più precocemente avviene una ripresa funzionale che risulta essere migliore rispetto a quanto avviene nei ratti reimpiantati più tardivamente.
CONCLUSIONI: Il tessuto tiroideo reimpiantato in una sede ectopica è capace di attecchire e riprendere completamente la sua funzionalità se mantenuto vitale da un adeguato mezzo di conservazione.

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