Verification of the feasibility of autogenous testis implant in omentum and abdominal wall in mice

Verificação da viabilidade do transplante autógeno de testículo no omento e na parede abdominal em ratos

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A B S T R A C T

Objective: To verify the feasibility of autologous transplantation of testes to the abdominal wall and omentum of rats without vascular anastomosis, analyzing the histological structure of the testicular cells after implantation. **Methods**: We used 60 male Wistar rats, 10-12 weeks of age, which were divided into three groups: control group: 20 rats without orchiectomy with sham operation; group 2: 20 rats undergoing bilateral orchiectomy, with one of the testicles being implanted into the greater omentum; and group 3: 20 rats submitted to bilateral orchiectomy, with one testicle implanted in the abdominal wall. After two months they were euthanized and the testes evaluated by histopathology. **Results**: the weight of the implants had a loss of 0.62 g in group 2, 0.73 g in group 3, whilst in the control group testes increased by 0.1 g. In pathological studies, the testicular structure was preserved in the control group; in group 2 there was 80% of inflammation and necrosis, Sertoli and Leydig cells were not visualized, and seminiferous tubules were found in two animals; in group 3 we found 75% of inflammation and a 60% necrosis, Sertoli cells could be visualized in only one specimen, while Leydig cells were seen in three. **Conclusion**: autologous transplantation of testis to the greater omentum and abdominal wall without vascular anastomosis is not viable in rats.

Key words: Testis. Abdominal wall. Omentum. Cryptorchidism. Transplantation, autologous.

INTRODUCTION

Cryptorchidism is synonymous with non-descended testicles. This circumstance is the retention of the testis in the abdominal cavity or the inguinal canal and it is found in around 1% of one-year-old children. Physiologically, testicular descent occurs in two distinct phases, morphologically and hormonally. During the firt, the transabdominal one, the testicles descend into the lower abdomen. It is believed that this phase is controlled by the hormone called mullerian inhibiting substance. During the second, the inguinoescrotal phase, the testes descend through the inguinal canal into the scrotum. This phase is dependent on androgen hormones. It is still not well-understood and it may be related to anatomical or hormonal features or to malformation syndromes associated with chromosomal aberrations[1,2].

Surgical treatment is the orchiopexy, which consists of surgical testicular allocation in the scrotum. This method may be infeasible when the pedicle of the testicle is not long enough for it to descend into the scrotum. In the inguinal canal, the testis is particularly exposed to trauma and crushing against the ligaments and bones, as well as presenting greater risk of testicular cancer than the descended testicle. The transplantation of autologous testis is an alternative for the treatment of cryptorchidism. The transplant accomplished by microvascular anastomosis of the spermatic vessels with the inferior epigastric vessels presents conflicting results, with testicular atrophy rates of 50%[2,3].

The testicular autologous transplantation without vascular anastomosis relies on the neovascularization of the implant and its functional viability in hormone production. This is substantiated by the fact that autotransplantation of other endocrine organs, such as spleen and ovaries, has proven feasible. It is valid to assume, therefore, that this transplant may become a treatment of cryptorchidism in the future. It is noteworthy that there is no systematic study to evaluate whether this technique is possible or not[3,4].

This study aims to verify the feasibility of autologous transplantation of testes in the abdominal wall

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and into the greater omentum of rats without vascular anastomosis, analyzing the histological structure of the testicular cells after implantation.

METHODS

The design of this study was approved by the Ethics and Research Committee of the President Antônio Carlos University (Protocol 180/07).

The study consisted of three groups of rats, each with 20 animals, with 10-12 weeks of age, thus divided: control group: 20 rats without orchiectomy with sham operation; group 2: 20 rats with bilateral orchiectomy and one testicle implanted in the greater omentum; group 3: 20 rats with bilateral orchiectomy and one testicle implanted in the abdominal wall. After two months they were euthanized and the testes evaluated by pathological examination.

The animals of the first group (control) underwent opening of the scrotum, externalization and manipulation of the testicles and attachments; after this maneuver they were put back in the scrotum, and the scrotal incision was sutured. The second group of animals underwent bilateral orchiectomy, follwoed by removal of the right testicle albuginea and immediate implant of the testicle in the abdominal wall. The third group was subjected to the same procedure of second group, but the implantation was performed in the greater omentum.

I the implantation laparotomy, we made a 3cm incision in the midline of the abdomen and implanted the testicle removed from the scrotum in the abdominal wall (group 3) and omentum (group 2), with single absorbable sutures. The implant in the abdominal wall was placed between the muscle layer and the subcutaneous tissue to the right of midline. The omental implant, on its turn, was placed on the ventral surface of the structure.

The selection of animals were casual after determining the weight and age of each. We included animals between 150 and 210g and between eight and nine weeks old. They were weighed after anesthesia and at the end of the experiment. The implanted testicles were weighed before and after resection, the testes of the control group were weighed when removed.

The animals were anesthetized with ketamine at a dose of 0.3 ml per animal. The surgical procedures were performed under aseptic conditions, with precautions in order to the animals recover uneventfully. All animals, whether in the control group or in the experiment ones, were followed for 60 days to allow time for the implant to adapt to the new site. At the end of this period, all rats were euthanized by ketamine overdose.

During the autopsy, we removed the right testicle from each animal in the control group, as well as the implanted testicle tissue from the animals that underwent autogenous implantation. The tissues removed in this process were weighed and fixed in 10% formalin for pathological examination. In the laboratory, the tissue fragments were stained with hematoxylin-eosin after dehydration and paraffin embedding. The slides were examined by two pathologists in a blinded fashion by analyzing the following: necrosis, acute and chronic inflammation, cell granulomatous calcification, neovascularization, elongated spermatids, primary spermatocyte, Sertoli cells, intact seminiferous tubules, epididymis, Leydig cells and other. The results of the pathological examination of the specimens and the comments made about the animals in the course of the experiment were recorded on a form designed specifically for the study.

Frequency distributions were constructed, and averages, standard deviations, and percentages indicated for each variable were calculated. Comparisons of data obtained from the experimental groups were made in RxC-type contingency tables and one-way ANOVA tables. Measurement of the statistical significance of comparisons was performed using chi-square or Fischer table for ANOVA or Kruskal-Wallis test for comparison of ordered sets of values. The degree of statistical significance in the analysis was 5%.

RESULTS

The average weight of the rats before the experiment was 207.5 g, with standard deviation (SD) of 30.8, varying weights between the minimum of 154g and the maximum of 308g. At the end of the experiment, the rats had a mean weight of 300.5 g, 28.1 g SD, and minimum and maximum weight of 288g and 399g, respectively.

The testes implanted had an average weight of 1.0 g with 0.16 SD and minimum and maximum weights of 0.7 g and 1.4 g, respectively. The testicular material withdrawn for pathological studies showed an average weight of 1.2 g, SD 1.18, minimum weight of 0.03g and maximum weight of 4.3 g. The testes of the control group had the average weight of 2.6 g, with 0.74 SD at the end of the experiment.

Table 1 compares the mean and SD of the difference in weights of mice, together with the mean and SD of the difference in weights of the testes, obtained between the beginning and end of the experiment, with the respective implantation sites. Concurrently, results of the respective values of p (probability) and the Kruskal-Wallis test values are also presented.

Table 2 shows the frequency of testicular histology comparing the autogenous implants groups with the control group. It also depicts the results of the statistical significance test each comparison was submitted to and the respective values of p (probability) and chi-square.

Table 1 - Mean and SD of the difference in weights of rats and testicles in the pre and postoperative period according to implant locations.

Implant site/	Control		Abdominal wall		Omentum		p	H*
difference in weights	Average	SD	Average	SD	Average	SD		
Difference of testicles weight	...	...	-0.81	0.20	-0.23	0.61	<0.001	11.989
Difference of rats weight	112	49.57	87.3	24.30	79.6	19.83	0.0440	6.246

** Kruskal-Wallis test*

Table 2- Comparison of experimental groups according to the frequencies of testicular histological characteristics.

Implant site/		Control		Abdominal wall		Omentum		P	X^2
Testicular structures		N	%	N	%	N	%		
Elongated Spermatid	Yes	20	100	0	0.0	0	0.0	<0.001	60.0
	No	0	0.0	20	100	20	100		
Primary Spermatocyte	Yes	20	100	0	0.0	0	0.0	<0.001	60.0
	No	0	0.0	20	100	20	100		
Sertoli cells	Yes	20	100	0	0.0	0	0.0	<0.001	60.0
	No	0	0.0	20	100	20	100		
Intact Seminiferous tubules	Yes	20	100	1	5	1	5	<0.001	51.8
	No	0	0.0	19	95	19	95		
Epididymis	Yes	19	95	0	0.0	1	5	<0.001	51.4
	No	1	5	20	100	19	95		
Leydig cells	Yes	20	100	2	10	0	0.0	<0.001	52.2
	No	0	0.0	18	90	20	100		

Table 3 shows the frequency of histological changes found in the implanted groups. It also shows the values of the relative risk and confidence intervals and p value obtained using Fisher's exact test.

DISCUSSION

The study was designed with young animals to simulate the period in which cryptorchidism is prevalent, ie

Table 3 - Comparison of the experimental groups according to the frequencies of histological changes observed.

Implant site/		Control		Abdominal wall		RR	CI 95%	P*
lesions found		N	%	N	%			
Necrosis	Yes	16	80	12	60	0.500	0.17- 1.39	0.150
	No	4	20	8	40			
Acute inflammation	Yes	7	35	2	10	0.722	0.50- 1.02	0.063
	No	13	65	18	90			
Chronic inflammation	Yes	16	80	15	75	0.800	0.25- 2.55	0.500
	No	4	20	5	25			
Granulomatous cells	Yes	5	25	1	5	0.789	0.60- 1.03	0.090
	No	15	75	19	95			
Calcification	Yes	14	70	9	45	0.545	0.25- 1.18	0.100
	No	16	30	11	55			
Neovascularization	Yes	7	35	5	25	0.866	0.57- 1.30	0.365
	No	13	65	15	75			
Other	Yes	10	50	2	10	0.555	0.35- 0.88	0.006
	No	10	50	18	90			

population of one-year-old children[3]. Experiments designed to study the effect of autologous organs have used a number of mice ranging from 30 to 80 and generally the amount of animals included in each study has been sufficient to establish the desired results. In this study we used 60 animals, with an average higher than similar studies. This quantity was sufficient to demonstrate that the type of transplant tested seems devoid of sustainability. The division of the groups was made by the influence of the studies cited in the literature that analyzed and reported success with implantation of autologous organs.

Cryptorchidism is the complete or incomplete absence of descent of intraabdominal testis into the scrotum. It usually occurs as an isolated disease but may be accompanied by other abnormalities of the genitourinary tract[3]. Cryptorchidism (gr. Kryptos, hidden) occurs in up to 30% of premature boys and about 3% to 4% of full-term ones[1]. The cause of cryptorchidism may be related to anatomical or hormonal features or malformation syndromes associated with chromosomal aberrations, the majority of patients having short spermatic vessels or persistence of the peritoneovaginal conduit as the cause[2-4].

The animals studied here showed no anomalies or anatomical malformations. All had intact scrotal and testicular structure at baseline. The implant sites were also presented in proper anatomic conditions. The histopathologic results of the control group showed that there were no factors but the autogenous implant and its consequential amendments that have influenced results.

When the testicle is in the inguinal canal, it is particularly exposed to trauma and crushing against the ligaments and bone structures. Moreover, the difference of two to three degrees of temperature between the scrotum and abdomen makes the testicular descent to the scrotum necessary so the sperm is fertile. It is observed that the two major problems associated with absence of testicular descent are infertility and increased risk of tumor development. The scrotum acts as a heat regulator for the testes, which are maintained approximately at 1° C below body temperature. The spermatogenic cells are sensitive to body temperature. In the study by Marc Luetjens *et al.*[5] on autologous testes transplants in nonhuman primates, the temperature of the implant site and its ability to produce neovascularization were taken into consideration, which have contributed to the success of the transplant, since spermatogenesis and hormone levels were maintained. Pettersson *et al.*[6] showed microscopic changes in the organ with migration anomaly in children two years of age[7-10].

Upon histological evaluation of the implanted testes we did not observe presence of spermatogenic cells (primary spermatocytes, elongated spermatids) at any implan. These cells are sensitive to body temperature. In the control group these cells were found in all slides.

It is estimated that patients with cryptorchidism have higher risk (about 10%) of developing tumors when compared to normal patients[1-7,11-13]. Studies by Petterson *et al.*[6] and Dias Neto *et al.*[8] describe that despite the early conduct of correcting cryptorchidism, this history remains a risk factor. Cryptorchidism was the factor most consistently associated with testicular tumor, found in 28.5% of seminomas and 15.5% of non-seminomas[2].

There were no cells with characteristics of testicular malignancy in the implanted tissues, probably due to the lack of tissue revascularization. The same was observed in the histological analysis of the control group, in which, despite the operative manipulation, the testicle remained histologically intact. The control group was not submitted to anatomical features of an ectopic testis, getting free of tumor development in the study.

It is known that ovarian grafts are feasible. The autologous ovarian transplants performed in the subcutaneous tissue of the inguinal region, next to the femoral vessels, showed 100% efficacy for histological analysis[8]. Nance *et al.*[13] compared uni to bilateral ovarian transplants in rats and observed that, although they displayed hormone production, the rats underwent cyclical changes in estrogen and levels of follicle stimulating hormone and luteinizing hormone, suggesting that the endocrine control mechanisms were altered but the histological structure was maintained[13]. Gosden *et al.*[12] performed ovarian grafts of sheep in mice and observed that the ovarian tissue not only were viable, but they presented follicles in various stages of development[12]. GuGasçna *et al.*[11] performed fresh and cryopreserved murine ovarian tissue allotransplantation and demonstrated maintenance of the endocrine function of the grafts, verified by the recovery of estrogen cycle[10]. The performance of autologous spleen implantation is a effective medical practice which is an alternative used as maintenance of spleen cells in patients undergoing splenectomy. Several studies have shown that the implantation of autologous spleen cells have the ability to maintain their phagocytic fuction[14-22].

When comparing the current study with published results for the autologous implants of ovaries and spleen, it is noteworthy that the histological structures of different endocrine organs behave in different ways for the implant without vascular anastomosis[23]. Marc Luetjens *et al* [5] and Pettersson *et al*[6] observed that, regardless of where the ovaries were implanted, the maintenance of their histological and endocrine structure is a rule in autogenous implants[5-6]. The same is true in relation to the spleen implant, which maintained its phagocytic function in the places where it was implanted in the studies.

In this study the testicular autologous transplantation to the omentum and abdominal wall showed histological lesions in high prevalence. The study by Wyns *et al.*[24] showed good results after cryopreservation of gonadal tissue in mammalian and reimplantation model in immunodeficient mice. Here there was aggression against the testis, possibly triggered by foreign body-type inflammatory response. It is therefore necessary to re-

evaluate the technique and add in its steps the use of immunosuppressants, with the aim of reducing the immune response to the ectopically implanted testicles. Even the omentum being highly vascularized, there was no statistical significance when comparing the presence of lesion to the implant site. The main factors for failure were lack of vascularization and increased sensitivity of testicular structures to the foreign body-like reaction[25,26].

Orchiectomy leads to weight gain in rats regardless of the time[13]. The cited work used 60 Wistar rats, which were evaluated for three months, with results divergent from those found in this experiment when it comes to weight gain. The control group showed greater weight difference, on average, when compared to the groups undergoing autologous implant. Even showing no statistical significance ($p < 0.05$) the differences of means may be considered to be caused by the process of aggression unleashed by the implant, not sustained by the control group, in contrast with the experiment in comparison.

In the study of Srougi *et al.*[15] 80 testicular isografts were performed in Lewis rats, with preservation of spermatic vessels and small segment of the aorta and vena cava from the donor animal. Revascularization of the graft testis was obtained by microsurgical anastomosis of the aorta and the vena cava of the donor and recipient animals. Surgical exploration and subsequent radiological studies confirmed the integrity of the grafts, confirming the efficiency of the presented method for obtaining isotransplantations of testis in rats[14].

The reported success with the isograft using the technique of microvascular anastomosis in the experiment above was not obtained in this work. The study had a high rate of degenerative lesions, such as necrosis (60% of the implanted testes in the abdominal wall and 80% of the implants in the omentum), acute inflammatory reaction (10% of the implants in the abdominal wall and 35% of the implants in the omentum), chronic inflammatory reaction (75% of the implants in the abdominal wall and 80% of the implants in the omentum), presence of granulomatous cells (5% of implants in the abdominal wall and 25% of the implants in the omentum) and calcification (70% of the implants in the omentum and 45% of the implants in the abdominal wall) and the relative absence of testicular cells such as elongated spermatids, primary spermatocytes, Sertoli cells, Leydig cells, intact seminiferous tubules in the testis and epididymis, showing the non-viability of the implant, the preservation of vascular structures being its major factor. Even being an isograft, subject to a higher rate of rejection, the cited work succeeded in its experiment, mainly explained by the maintenance of the vascular network.

Feria *et al.*[16] reported a case of an eight year old boy with bilateral cryptorchidism in whom they practiced intraabdominal autogenous transplanting of one testis using microvascular technique[15], with satisfactory results.

By failing to present results showing the viability of the autogenous testis implant in contrast to ovary and spleen implants, testis transplant without vascular anastomosis was not feasible.

In conclusion, autologous transplantation without vascular testis to the omentum and abdominal wall of Wistar rats is not viable.

RESUMO

***Objetivo**: verificar a viabilidade do transplante autógeno de testículos na parede abdominal e omento, em ratos, sem anastomose vascular, analisando a estrutura histológica das células testiculares após o implante. **Métodos**: foram utilizados 60 ratos Wistar, machos, de 10-12 semanas de idade, distribuídos em três grupos: grupo controle: 20 ratos sem orquiectomia, com operação simulada; grupo 2: 20 ratos com orquiectomia bilateral sendo um testículo implantado no omento maior; grupo 3: 20 ratos com orquiectomia bilateral, sendo um testículo implantado na parede abdominal. Após dois meses eles foram mortos e os testículos avaliados pelo exame anatomopatológico. **Resultado**: o peso dos implantes teve perda de 0,62g no grupo 2, de 0,73g no grupo 3 e no grupo controle houve aumento de 0,1g. Ao estudo anatomopatológico, no grupo controle a estrutura testicular foi preservada; no grupo 2 encontrou-se 80% de inflamação e necrose, não foram visualizadas células de Sertoli ou de Leydig, em dois animais encontraram-se túbulos seminíferos; no grupo 3 encontrou-se 75% de inflamação e 60% de necrose, somente em um conseguiu-se visualizar células de Sertoli e em três células de Leydig. **Conclusão**: não é viável o transplante autógeno de testículo sem anastomose vascular em ratos no omento maior e na parede abdominal.*

***Descritores:** Testículo. Parede abdominal. Omento. Criptorquidismo. Transplante autólogo.*

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