Testicular histology after intestinal pedicle flap (cecum) apposition in rats[1]

Histologia testicular depois da aposição de um retalho intestinal (ceco) em ratos

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ABSTRACT

Purpose: Histological study of vascularization between a cecal pedicle flap and the testicle of Wistar rats. **Methods**: Fifty-three rats were studied. G1: submitted to celiotomy (a), mobilization of the right testicle (RT) to the abdomen (b), cecal flap suture to the RT (d) and cavity closure. G1: procedures a, b and d and fixation of RT into abdomen. G3: procedures a, b and d, exposition of RT to air and reposition into scrotum. G4: not operated. Euthanasia and histology was done after 20 days. Histometry and lesions score classification was done. Testicular vascularization was studied with comparison between G1 and G3. A $p < .05$ was considered significant. **Results**: The G1 RT diameters were not different to G2 RT and all have decreased size in comparison with RT of G3 and G4. The lesions score in the RT was 5.83 in G1 and 3.3 in G2 without statistical difference. The vascularization's average in G1 was 16.9 vessels in 400X field in the RT. In the G3 this average was 0.96 to the RT and 0.92 to left testicles. The weight's average in G1 was similar with G2 but different of G3 and G4. **Conclusion**: A significant increase of vascularization was observed between the intestinal flap and the rat testicle.

Key words: Testis. Surgical Flaps. Histology. Rats.

RESUMO

Objetivos: Estudar histologicamente a vascularização entre um retalho cecal e o testículo de ratos Wistar. **Métodos**: Cinquenta e três ratos foram estudados. G1, submetidos a (a) celiotomia, (b) mobilização do testículo direito (TD) para o abdome, (c) sutura do retalho cecal ao TD, (d) fechamento da cavidade. G2, procedimentos (a, b e d), com o TD fixado no abdome. G3, procedimentos (a, b e d), com exposição do TD ao ar e retorno ao escroto. G4 não operados. Após 20 dias, eutanásia e histologia. Realizou-se histometria e classificação segundo escore de lesão. Avaliou-se a vascularização testicular, comparando-se os grupos 1 e 3. Considerou-se significativo um $p < 0,05$. **Resultados**: Diâmetros dos TD no G1, iguais ao G2 e diferentes de G3 e G4. O escore de lesão nos TD foi de 5,83 pontos para o G1, de 3,3 pontos para o G2, não havendo diferença significativa, porém diferentes de G3 e G4 (sem lesão). A vascularização no G1 teve média de 16,9 vasos por campo de grande aumento no TD. No G3 a média foi de 0,96 no TD e 0,92 no TE, com diferença significativa. O peso médio do G1 foi igual ao G2 e diferente de G3 e G4. **Conclusão**: Houve aumento significativo da vascularização entre o retalho e o testículo do rato.

Descritores: Testículo. Retalhos Cirúrgicos. Histologia. Ratos.

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Introduction

Undescended testis, also called cryptorchidism, is characterized by the absence of the testicle in the scrotum. It is usually due to failure in embryogenesis or in male gonad migration. This condition may also occur due to ischemia or gonadal atrophy[1-3]. The incidence is 33% in premature infants, 4% to 5% in live mature births and 0.8% to 1% at the end of the first year of life. Currently, orchidopexy is proposed between 6 months and 12 months of age to bring the gonad down into the scrotum[4,5]. Several efforts have been made to preserve gonad blood flow when performing this kind of procedure, mainly in the cases when it is necessary to ligate the testicular pedicle. One of the possible solutions would be to induce better testicular vascularization, which could increase gonadal blood flow.

The goal of this experimental study is to evaluate, histologically, the testicular vascularization induced by an cecal pedicle flap, sutured to the testicles of Wistar rats and study the testicular lesions resulting from this procedure. We are especially interested in the ideal way (technically) to perform the intestinal apposition to the testicle and induce vascular proliferation. In this experiment we do not seek to prove that increased vascularization found is sufficient to maintain good blood flow to the gonad.

Methods

This experiment was approved by the CEUA (Ethics Commission for the Use of Animals) of the Federal University of Uberlândia (UFU).

Fifty-three adult male Wistar rats (*Rattus norvergicus albinus, Rodentia mammalia*), aged between 12 and 16 months, apparently healthy, and weighing between 290g and 440g were randomly selected for this study. Seven were excluded after surgical procedures. The animals were supplied by the Experimental Surgery Laboratory of the Operative Techniques, Division of the Medical School, Federal University of Uberlândia (UFU), and were appropriately acclimatized in an experimental environment. They were submitted to a preoperative solid food fasting for 12 hours and weighed thirty minutes before the surgical procedures A Filizola® balance was used, from 0 to 20Kg and minimal interval of 20g.

Anesthesia was performed with a subcutaneous injection of Cetamine hydrochloride (general anesthetic), using a 0.4 mg /100g dose, in association with a 2% Xylazine hydrochloride solution, with a dose of 0.1mL /100g dose (anesthetic, analgesic and muscle relaxant), according to Laboratory routine and similar to that approved at other experimental laboratories[6,7]. The animals were placed in operative supine position after abdominal hair removal by surgical shaving.

Antisepsis was done with a 2% alcoholic polyvinylpyrrolidone iodine solution and a sterile window drape was placed on the shaved area as an aseptic procedure. All the procedures were done following operative techniques of asepsis and antisepsis, including surgical gowns, gloves, masks and caps. The animals were divided into four groups: 12 animals in G1, 12 in G2, and 11 in G3 and 11 in G4, a total of 46 rats.

Pilot study

The original idea was to perform an intestinal flap suture to the gonad, without the mucosal layer, and then replace the testicle back into the scrotum. However, it was impossible to take out the mucosa without provoking flap ischemia. Thus, as we learned in the pilot study, we decided to maintain the mucosa and observe the histological changes resulting from this procedure. A cecal flap was used, and it was impossible to return the testicle to the scrotum, therefore we created a control group (G2) with the testicle placed into the abdomen.

Surgical procedures

Group 1: The rats were submitted to an approximately 3cm-long celiotomy, mobilization of the right testicle to the abdomen by traction. Identification of the cecum, preparation of the cecal flap from a distal area (1 cm) and flap apposition to the right testicle, with a continuous suture of polygalactin-910 (Vicryl®) 6-0 (Figure 1). The mucosal surface of the flap was placed in contact with the testicle (albuginea) (Figure 2). Viscera were replaced and the cavity was closed, after testing the suture with a "tire test", with a two-layer suture of polypropylene (Prolene®) 4-0.

Group 2: Submitted to celiotomy, mobilization of the right testicle to the abdomen by traction, like in Group 1. The right testicles were fixed in the abdomen (to the peritoneum) with a transfixing suture of polypropylene (Prolene®) 6-0, viscera replaced and cavity closed. This group was used as a Group 1 control, where the right testicles were placed into abdomen.

Group 3: Submitted to celiotomy, mobilization of the right testicle to the abdomen by traction. The right testicle was exposed to air for 3 minutes and was replaced in the scrotum. The viscera were replaced and the cavity was closed.

Group 4: The rats were not operated on (control), only separated and observed, as in previous groups and sacrificed for histological evaluation after 20 days.

After surgical procedures the animals were treated with the same chow (Agroceres®), *ad libitum*, and placed in same characteristics environments (cages with food and water).

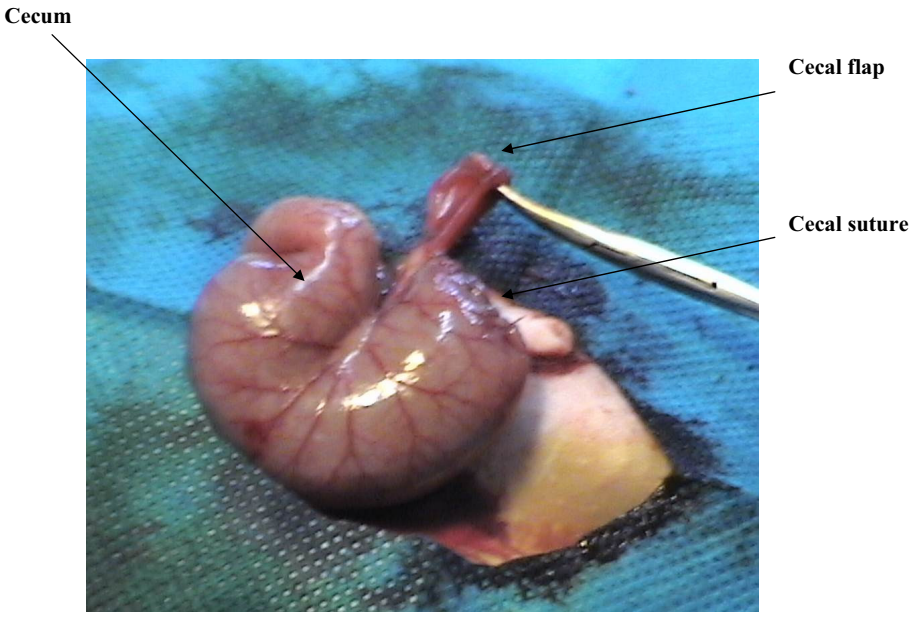


FIGURE 1 - Photograph of preparation of the cecal flap

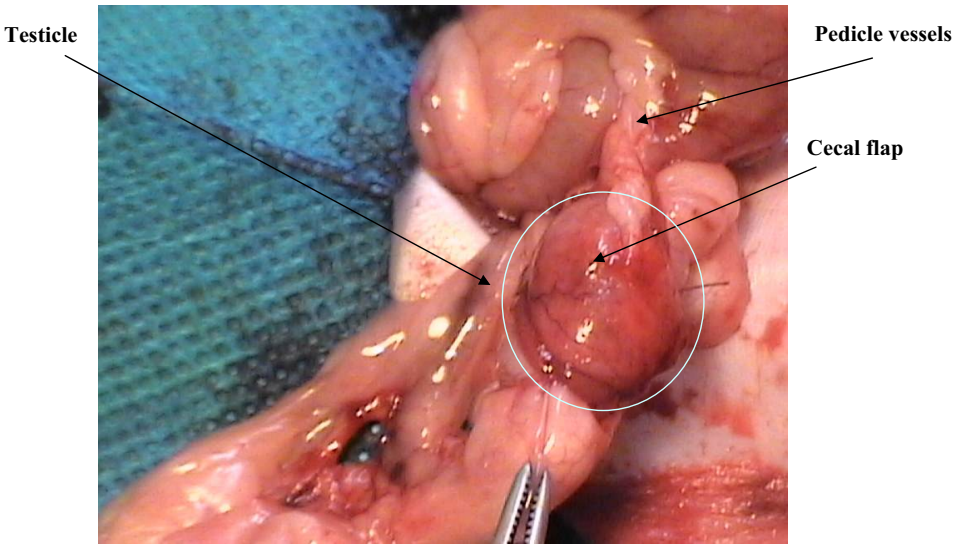


FIGURE 2 - Photograph of sutured cecal flap to testicle

Twenty days after the surgical procedures (Groups 1, 2, 3 and selection of the Group 4) the animals were weighed again and euthanasia was performed with lethal doses of Cetamine hydrochloride (16mg/100g).

Both testicles of each animal, of all groups were extirpated, placed in flasks with 10% formalin solution, appropriately identified and sent for histological analysis. The pathologist was blind to the groups, although it was clear which testicles included an intestinal flap.

At the University (UFU) Pathology Laboratory, the testicles were dehydrated in ethanol, diaphanized in xylol and prepared in a paraffin tissue block. The histological sections of 3µm was done and stained by Hematoxilin-Eosin (HE) and Masson trichromic, for better identification of fibrosis, when necessary. The secctions were evaluated by Common Optical Microscopy (Olympus® CH-2 de 40X a 1000X) and the largest diameters were measured in 40 x augmentation. The folowing characteristics were analyzed in the hystological study: albuginea thickness, seminigerous tubules diameters, presence of different tipes of cells, peritubular fibrosis and necrosis by coagulation or inflammation.

A morphometric analysis (histometry) and a classification according to testicular lesion score, based on Johansen's score, apud Mclachlan[8], were done. The largest testicle diameters were measured, and lesions were studied and classified from 0 (histologically normal) to 8 (destruction of the whole testicular parenchyma caused by necrosis and/or inflammation) (Chart 1).

CHART 1 - Lesions score classification found on rats testicles after surgical manipulation

SCORE	LESIONS
0	Absence of damage: seminiferous tubules presenting uniformly preserved diameter, patent flaming, presence of all cells of germinative lineage, with active spermatogenesis and absence of fibrosis, necrosis and inflammation;
2	Testicular damage characterized by thickening of the albuginea tunic, reduction of tubular diameters with accentuated depletion of germinal cells of most of the tubules and absence of necrosis and inflammation;
4	Testicular damage characterized by thickening of albuginea tunic, reduction of tubular diameters with accentuated depletion of the germinal cells and destruction of some tubules (less than half) by necrosis and or inflammation;
6	Testicular damage with destruction of most of the testicular parenchyma by necrosis and or inflammation;
8	Destruction of the whole testicular parenchyma by necrosis and or inflammation

Testicular vascularization was evaluated after the identification of vessels between the cecal flap and right testicle, by microscopy, in five fields of 400X magnification. The Group 1 right testicle vascularization was compared with Group 3 right and left testicle vascularization, considered ideal control.

Tukey tests were used for comparison between averages and Mann-Whitney to evaluate vascularization (Bioestat). The Kruskal-Wallis test was used to compare the lesion scores (Sisvar). A $p < .05$ was considered statistically significant. The statistical programs used, Biostat and Sisvar, are free license software.

Results

Seven rats were excluded from the study. Four rats from Group 1 and one from Group 3 died and the main cause detected was infection (peritonitis) with intestinal obstruction. One rat was excluded because it was impossible to anesthetize it (G2) and another because of suppuration in the scrotum (right side). Therefore, the testicles of 46 rats (92 testicles) were histologically analyzed.

Testicular diameters

The averages of the largest longitudinal and transverse diameters, in each group were evaluated morphometrically (Table 1).

In Group 1, the average longitudinal diameter was 12.75mm in the right testicles (RTLD) and 16.54mm in the left testicles (LTLD). The average transverse diameter in Group 1 was 6.65mm for the right testicles (RTTD) and 9.17mm for the left testicles (LTTD).

A statistically significant reduction was observed (Tukey, $p < .05$) in the diameters of the operated right side testicles from Group 1, compared to the right ones from Group 3 exposed to the air and the right ones from Group 4, which were not operated on.

In Group 2, the longitudinal diameters of the right testicles were 11.41mm. On the left side, the average was 17.31mm. The average testicular transverse diameter in Group 2 was 5.82mm among the right testicles and 9.6mm among the left ones (Figure 3).

TABLE 1 - Average and standard deviations of testicular diameters in the four groups

Groups	G1 Average (s)	G2 average (s)	G3 average (s)	G4 average (s)
LDRT (s)	**12.75mm** (± 5. 26)	**11.41mm** (±1.44)	17.4mm (± 0.69)	17.04mm (± 0.78)
LDLT (s)	16.54mm (±2.25)	17.31mm (± 0.84)	17.59mm (± 0.99)	16.39mm (± 2.31)
TDRT (s)	**6.65mm** (± 3.65)	**5.82mm** (± 0.74)	8.84mm (± 0.53)	9.04mm (± 0.45)
TDLT (s)	9.17mm (±1.12)	9.60mm (± 0.45)	9.12mm (± 0.57)	9.11mm (± 0.54)

Legend:
LDRT = Longitudinal Diameter of Right Testicle;
LDLT = Longitudinal Diameter of Left Testicle.
TDRT = Traverse Diameter of Right Testicle;
TDLT = Traverse Diameter of Left Testicle.
(s) = Standard Deviation.

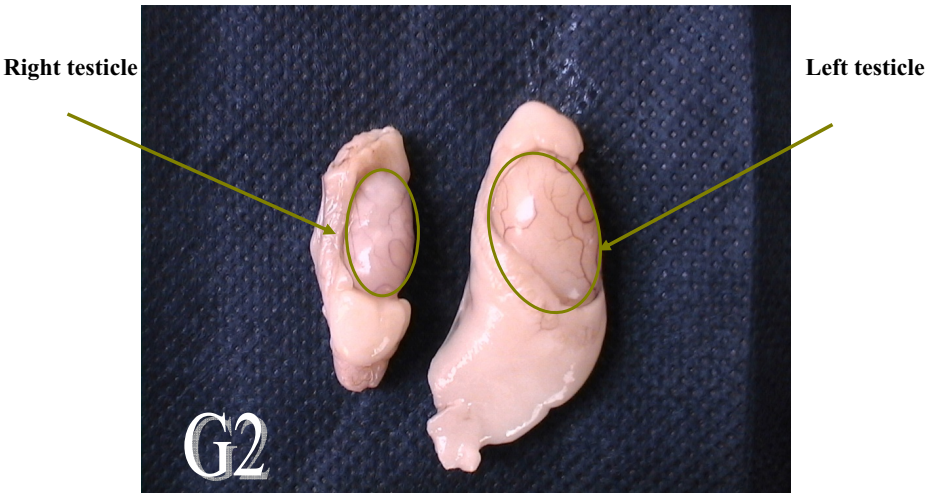


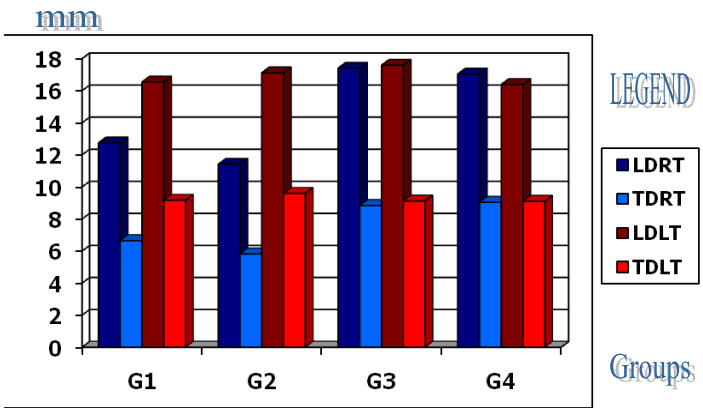
FIGURE 3 - Right and left testicles of one of the rats from Group 2 with different diameters

In the Group 2, the diameters of the right testicles operated on, were also statistically smaller (Tukey, $p < .05$) than those of the right testicles from Group 3 and right testicles from Group 4 (not operated on).

In Group 3, the average longitudinal diameter of the right testicles (exposed) was 17.4mm and 17.59mm for the same diameter in the left ones. The average transverse diameter of the right testicles was 8.84mm and 9.12mm for the left ones, in Group 3.

In Group 3, the preservation of testicular diameters was observed without a statistical difference (Tukey, $p > .05$) when compared with the right testicle exposed to air (Group 3) and with those from Group 4 (not exposed).

In Group 4, the average longitudinal diameter of the right testicles was 17.04mm and 16.39mm in the left ones. The average transverse diameter of the right testicles was 9.04mm and 9.11mm, for those on the left. The preservation of the gonadal diameters was also observed, without a statistical difference (Tukey, $p>0.05$) when we compare right testicles from Group 4 with left ones from Group 4 and with those on the right side from Group 3 (Figure 4).



LDRT = Longitudinal Diameter of Right Testicle;
LDLT = Longitudinal Diameter of Left Testicle.
TDRT = Traverse Diameter of Right Testicle;
TDLT = Traverse Diameter of Left Testicle.

FIGURE 4 - Schematic representation of testicular diameters (mm) in the four groups

Testicular lesions

The testicular lesions score was greater in Group 1, where the testicle was placed in the abdominal cavity and sutured to an intestinal flap. In this group, an average of 5.83 points for the right testicles and 0.166 points for the left ones was calculated (only one testicle presented with score = 2).

In Group 2, in which the right testicle remained 20 days in the intra-abdominal position, there were also significant lesions with an average of 3.3 points for the right testicles and zero for the left ones, which were in the scrotum.

In groups 3 and 4 there were no testicular lesions (Figure 5). The statistical analysis in these groups shows that Group 1 does not differ statistically from Group 2 and both differ from Groups 3 and 4 (Kruskal - Wallis, $p<0.05$).

This means that the lesions that occurred in testicles with flaps (cecum) in Group 1 were similar to those that occurred in the testicles placed in the abdominal cavity (Group 2). The operation, which mobilizes the testicle, exposes it to the air and in the end puts it back into the scrotum, did not provoke any testicular lesion (Group 3) (Figure 6).

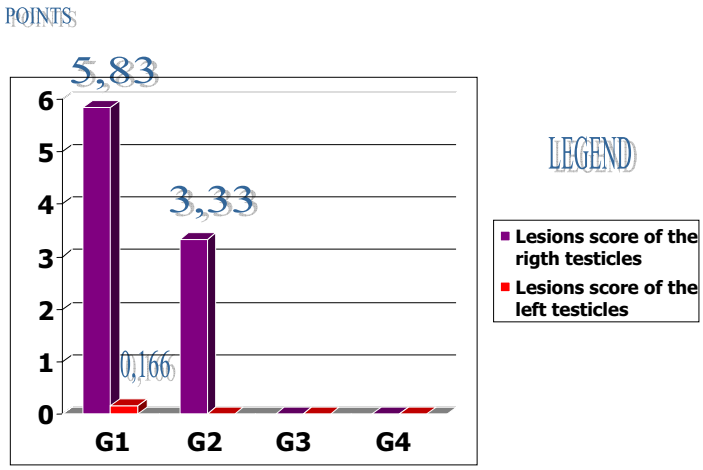


FIGURE 5 – Lesions score averages of each group

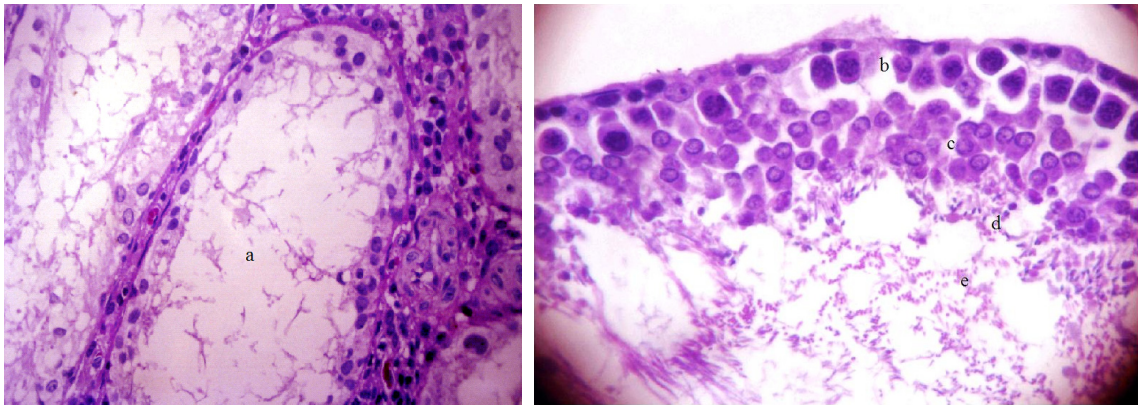


FIGURE 6 – Photomicrographs showing comparison between damaged testicle with score 4 in rat from Group 2, (HE – 400X), with marked germ cell depletion (a) and normal testicular histology with cellular preservation (b-espermatogonial, c-espermatocyte, d-spermatid, e-spermatozoid)

Weight

Tables 4 and 5 present the average weight (g) of the rats before and after the procedures, in each studied group. There was a small weight variation, when the weight before and after the procedures was compared (Table 2).

It was observed that when the averages weights were compared, there were differences among the groups. Group 3 (low average) was statistically the same as Group 1 and different from Group 2 and Group 4 (Tukey, $p < .05$). The weight variation did not influence the sizes of the left testicles of the rats in all groups (Figure 4).

TABLE 2 - Variations, averages and standard deviations of rats weights (g), in each group, before (w1) and after (w2) surgical procedures, before euthanasia

	WEIGHT IN GRAMA (w1, w2)			
GROUPS	G1	G2	G3	G4
WEIGHT VARIATION	w1 = 290g - 400g w2 = 300g - 400g	w1 = 290g - 420g w2 = 280g - 440g	w1 = 300g - 340g w2 = 300g - 400g	w1 =310g - 440g w2 = 310g - 420g
AVERAGE	w1 = 350g w2 = 352.5g	w1 = 355.41g w2 = 370.41g	w1 = 315.45g w2 = 339.09g	w1 = 364.54g w2 = 366.36g
STANDARD DEVIATION	w1 = 33.30 w2 = 41.80	w1 =35.38 w2 = 48.35	w1 = 16.94 w2 = 31.76	w1 = 38.82 w2 = 34.71

Vascularization

A histological evaluation was performed in the right testicles in 10 of 12 rats from Group 1. In two cases, this analysis was impaired due to the deterioration of the material. The same analysis was used in the right and left testicles from Group 3.

A 16.9 average vessel, in 5 fields of 400X magnification was observed for the testicles with intestinal flaps (G1). In Group 3, only a 0.96 average vessel, in the right testicles and a 0.92 average vessel, in the left testicles was observed, using the same magnification (Figure 7). There was a significant statistical difference among the groups compared (Group 1 and Group 3 - Mann - Witney, $P < .05$) (Table 3).

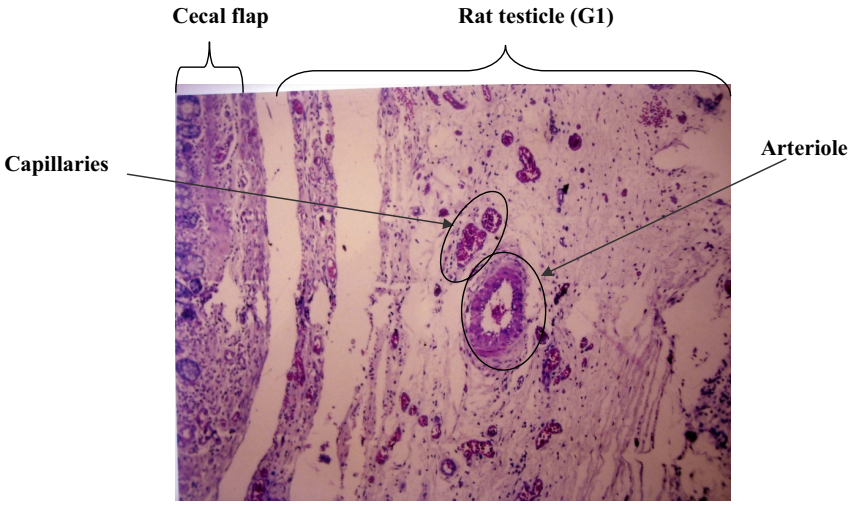


FIGURE 7 – Photomicrograph of details of transition area between the cecal flap and testicle with numerous capillaries and arteriole in Group 1 rats, (HE - 200X)

TABLE 3 - Average number of blood vessels (vv), in five large magnification fields (400X), in Groups 1 and 3 with respective standard deviations

GROUPS (variation) - VESSELS AVERAGE IN 5 LMF* (400X)		- STANDARD DEVIATIONS
G1 (4.8 a 30.4)	RT = 16.9 vv/5 LMF	± 8.360
G3 (0.8 a 1)	RT = 0.96 vv/5 LMF	± 0.080
G3 (0.8 a 1)	LT = 0.92 vv/5 LMF	± 0.100

*5CGA = Five larg magnification fields (400 X). RT (Right Testicle) LT (Left Testicle)

Discussion

Efforts to preserve testicular blood flow in patients with cryptorchidism, submitted to surgical treatment, have been mentioned in the literature, especially after Fowler-Stephens procedure introduction in 1959. Nowadays, the atrophy rates, resulting from testicular desvacularization, still high[9-11]. Autotransplantation was tried, but few results have been showed, especially in small children[12].

More recently, in the literature, efforts were observed to promote testicular revascularization by using omentum, bladder flaps or fasciovascular flaps sutured to the gonads in experimental models. These procedures have indicated a new way for reducing the rates of postoperative testicular atrophy[13-15].

The experiment results showed us, that it was easily possible to use an intestinal flap with its pedicle, without damage to the gross anatomy of the donor organ. The mobilization of a flap, with a long pedicle, to the scrotum can be very important in high position testicle surgical procedure. Some authors used bladder[14] and omentum[15-17] in their experiments. However, we should remember that orchidopexies in human beings have been increasingly indicated for very young boys, about nine months old[18]. At this age, the child's omentum is not highly developed and this condition could result in technical difficulties in descending the testicles into the scrotum. The bladder does not have a vascular pedicle that can be isolated and its wall should occupy the inguinal channel in the case of orchidopexy. In cases of bilateral cryptorchidism, there would not be enough bladder tissue for surgical correction. The instestinal flap could be a good option, to be evaluated in future studies, in which a long vascular pedicle will be necessary to replace the testicles in the scrotum (Fowler-Stephens orchidopexy).

An important observation was that in the testicular lesions variable, there was no significant difference between the right testicles, which were only mobilized and exposed to the air (Group 3) and the right testicles of Group 4, not operated on. According to these observations, handling the gonad or anesthesia and surgical procedures did not contribute to testicular lesions.

Another important fact detected in the experimental model, that placing the right testicles in the abdominal cavity produced the effect of gonadal atrophy with volume reduction in the right testicles of groups 1 and 2, a fact already mentioned in the literature[19]. The suture of the intestinal flap directly on the right testicle in Group 1 and the suture of the right testicle to the peritoneum in Group 2, associated with a rise in the temperature of the testicles positioned in the abdomen contributed to increase the lesion score of these two groups. The lesions in these groups have been larger than in other groups. These data are corroborated by authors who found a significant difference in testicular lesions when direct suturing was applied to the testicle compared with indirect fixation techniques[20,21].

Good adhesion of the intestinal flap to the testicle was observed in Group 1, and a statistically significant ($p < .05$) increased testicular vascularization, when we compared Group 1 (flap) and Group 3 (testicular mobilization).

Increased vascularization can be a step to preserve testicular blood flow. However, the findings in this experiment do not demonstrate that intestinal flaps could maintain an adequate testicular blood flow. This hypothesis should be tested in future research, on which Fowler-Stephens procedure must be done.

In a future application, in human beings, the presence of the intestinal mucosa should be avoided. This detail could contribute to a reduction in complications due to infection and neoplastic changes, similar to those which occur in bladder augmentations[22], using complete intestinal wall.

Another reason to avoid the mucosa layer is the unknown long term effects of the mucus in contact with the peritoneum and viscera.

For technical reasons a cecal flap was used in the experiment. However, it is well known that the best intestinal segment to produce intestinal pedicle flaps to replace the urinary tract, in humans, is the ileum, approximately 20cm to 30cm of the ileocecal valve, as already occurs in bladder augmentation or Mitrofannoff-Monti type intestinal conduits[23-25]. The fixation of the intestinal flap seems better when sutured around the terminal portion of the testicular vessels. This has already been mentioned in a publication where omentum was used as a neovascularization inductor[15-17].

Conclusion

A significant increase of vascularization was observed between the intestinal flap and the rat testicle.

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