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Quality and quantity comparison study of corrosion casts of bovine testis made using two synthetic kits: Plastogen G and Batson no. 17

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Background: *Although corrosion casting has been implemented for almost five centuries, the choice of resin has a strong influence on the effectiveness of casting vessel formations. Our aim was to compare quality and quantity features of two groups of corrosion casts made using two kits: Plastogen G resin and Batson no. 17 resin.*

Materials and methods: *Thirty corrosion casts were made of testicular arterial vessels (15 made using Plastogen G and 15 made using Batson no. 17) and their shape, colour, fragility and flexibility were evaluated. The following parameters were measured: maximal width of corrosion cast of testis, maximal length of centripetal and centrifugal arteries, diameter of testicular artery and its terminal branches. Based on these measurements, five indexes were calculated (Iq1–Iq5).*

Results: *Generally both groups of corrosion casts demonstrated similar attributes. Only the rami tunicales minores displayed higher fragility and lower flexibility in the Plastogen G group than the Batson no. 17 group. The only observed quantitative difference was that Iq3 was significantly lower in the Plastogen G group (0.71 ± 0.01) than in Batson no. 17 group (0.79 ± 0.01; $p = 0.0092$).*

Conclusions: *The two corrosion cast groups displayed similar qualitative and quantitative attributes. Batson no. 17 appears to be a good resin for three-dimensional visualisation of extra- and intratesticular arterial vessels.* (Folia Morphol 2019; 78, 3: 487–493)

Key words: corrosion cast, bovine, testis, technical notes, Batson no. 17, Plastogen G

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INTRODUCTION

The method of corrosion casting has been known since the 16th century, when Leonardo da Vinci made the first casts by injecting dissolved wax into bovine cerebral ventricles and heart chambers [5, 24]. Over the course of the following centuries, anatomists have improved the casting media, the method of injection, and the method of removing the surrounding tissues to produce a more accurate replica of the biological structures [5, 24]. Nowadays, several commercially-produced partially polymerised methacrylates are available, such as Mercox, Plastogen G, Trylon, Duracryl Plus, Technovit and Batson plastic no. 17 [3, 10, 12, 14, 18, 19].

Previous corrosion cast studies of the vascularisation of bovine testis using Plastogen G allowed quantity assessment to be made of anastomoses in the spermatic cord [22], as well as the first descriptions of anastomoses in the tunica albuginea [20] and the types of intratesticular arteries [21]. Unfortunately, there is a need to identify a new resin for use in future studies as Plastogen G is no longer produced and that's why we were looking for a new resin for our research. Also, Batson no. 17 has never been used in studies of bovine testis vascularisation.

Therefore, the present study compares key qualitative and quantitative aspects of two groups of corrosion casts made of two kits: Plastogen G and Batson no. 17. No such comprehensive comparison currently exists in the literature.

MATERIALS AND METHODS

Two groups of bovine testis corrosion casts were formed. During the first part of the study, casts were made using Batson no. 17 resin (group 1). In the second part, these specimens were compared with Plastogen G casts (group 2) made over the course of previous studies [3, 14, 18, 19, 20–22]. The second group of casts were stored in the collection of the Interfaculty Chair of Anatomy and Histology, Medical University of Lodz, Poland.

The research project was approved by the Bioethics Commission of the Medical University of Lodz (Protocol number ID: RNN/120/07/KE). All procedures took place in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

First stage

The bovine testes (Polish black and white Holstein–Friesian breed) were taken within 9 h after the animal had been slaughtered for butchering. None of the organs in either group showed any signs of pathological alteration or disease. No attempt was made to match morphological studies to age of the donor or position of the gonad (left or right) and material was analysed uniformly in each case. The testes were taken together with their coverings. The spermatic cord was isolated and prepared to find access to the testicular artery. In group 1, 15 specimens were prepared for access to the arterial vessels and study of the arterial network. A corrosive cast of the testicular vessels was obtained by the following process

First, 0.9% NaCl solution was injected into the chosen vessel and perfusion was maintain for 10–20 s in order to flush out possible clots. This saline perfusion was followed by injection of 10 mL of 3% glutaraldehyde solution in cocadylate buffer (pH 7.4). The testicular artery was then filled with synthetic resins Batson no. 17, and these were stained with red pigments. Following this, the gonad was left for 24 h in warm (20°C) water in order to toughen the resin. After toughening, the specimen was placed in 40% KOH solution (50°C) for 24 h to dissolve the organic parts. The remnants of the dissolved tissues were removed by continuous warm water flush for 24 h, followed by a short wash with water with a small amount of standard washing liquid, and then a final flush of distilled water. The casts were later dried out by air flow at room temperature for a suitable time.

Second stage

Both groups of corrosion casts, i.e. those cast in Batson no. 17 (n = 15) and those in Plastogen G (n = 15), were then compared. The casts were subjected to macroscopic observation with a stereoscopic binocular scope. Digital images were taken of the macroscopic observations for each specimen. The images were saved in jpeg format and later digitally transformed and analysed using CorelDRAW. After the photographic images were obtained, the diameters of selected vessels were measured with a micrometre digital calliper with an accuracy of 0.01 mm (Scala 150 mm 0.01 mm 230.207, Scala Messzeuge GmbH, Kelterstrasse, Germany).

Comparison study of the two groups of corrosion casts.

Table 1. Qualitative assessment the two corrosion cast groups

	Shape			Colour			Fragility			Flexibility		
	Testicular artery	Rami tunicales majores	Rami tunicales minores	Testicular artery	Rami tunicales majores	Rami tunicales minores	Testicular artery	Rami tunicales majores	Rami tunicales minores	Testicular artery	Rami tunicales majores	Rami tunicales minores
Plastogen G	Round	Round	Round	Shiny	Shiny	Matt or shiny	Solid	Solid	Brittle	Flexible	Flexible	Stiff
Batson no. 17	Round	Round	Round	Shiny	Shiny	Shiny	Solid	Solid	Solid	Flexible	Flexible	Flexible

The qualitative assessment included several features of the corrosion casts presented in Table 1:
- shape of testicular artery in transverse section before division on terminal branches (round or oval);
- shape of direct branches of the testicular artery named as rami tunicales majores in transverse section (round or oval);
- shape of branches arising from major branches in transverse section — named as rami tunicales minores (round or oval);
- colour of testicular artery, rami tunicales majores and minores (matt or shiny);
- fragility of corrosion cast of testicular artery, rami tunicales majores and minores (brittle or solid);
- flexibility of corrosion cast of testicular artery, rami tunicales majores and minores (flexible or stiff).

The qualitative assessment included fragility and flexibility was based on definition described by Doomernik et al. (2016) [4]. According to these definitions:
- fragility — a substance is brittle when damaged by minimal tissue manipulation;
- flexibility — a substance is flexible when it is easy to manipulate and behaves like the surrounding tissues during manipulation.

The quantitative assessment included several indexes presented in Table 2. The following parameters were measured (Figs. 1–4):
- maximal width of corrosion cast of testis measured in transverse section (Wt) (Fig. 1);
- maximal length of centripetal arteries (Lcpa) (Fig. 2);
- maximal length of centrifugal arteries (Lcfa) (Fig. 2);
- diameter of testicular artery leaving the spermatic cords on the posterior surface of the testis (Dta1) (Fig. 3);
- diameter of testicular artery before division into its terminal branches (Dta2) (Figs. 3, 4);
- diameter of rami tunicales majores after division of the testicular artery (Drtm) (Fig. 4).

The following indexes were calculated:

Table 2. Quantitative assessment of two groups of corrosion casts.

Parameter	Plastogen G	Batson no. 17
Wt [mm]	45.59 (42.97–56.48)	59.91 (50.86–64.72)
Lcpa [mm]	22.04 (20.36–24.98)	24.17 (23.32–33.86)
Lcfa [mm]	19.50 ± 2.99	23.32 ± 5.25
Dta1 [mm]	3.65 ± 0.47	4.10 ± 0.40
Dta2 [mm]	3.45 ± 0.53	4.53 ± 0.51
Drtm [mm]	2.79 (2.54–3.13)	3.41 (3.26–3.55)

Normally distributed data are presented as means with standard deviations. Non-normally distributed data are presented as medians with lower and upper quartile values; Wt — maximal width of corrosion cast of testis measured in transverse section; Lcpa — maximal length of centripetal arteries; Lcfa — maximal length of centrifugal arteries; Dta1 — diameter of testicular artery leaving the spermatic cords on the posterior surface of the testis; Dta2 — diameter of testicular artery at the level of division into its terminal branches; Drtm — diameter of rami tunicales majores after division of testicular artery

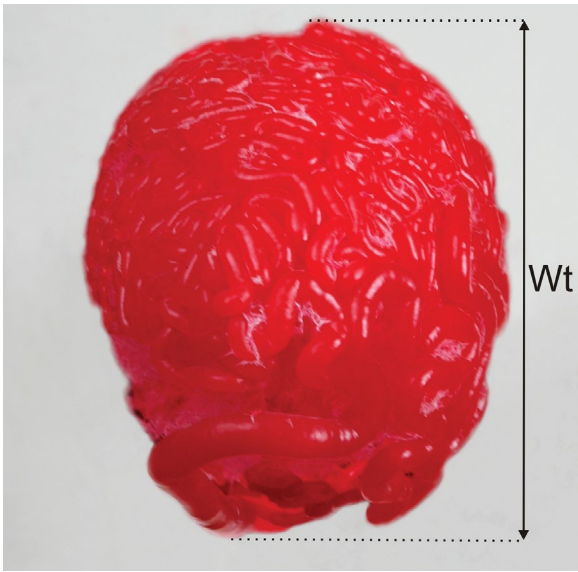

Figure 1. Corrosive cast of bovine blood vessels (Plastogen G); Wt — maximal width of corrosion cast of testis measured in transverse section.

- Iq1 = maximal length of centripetal arteries/maximal width of corrosion cast of testis (Lcpa/Wt);

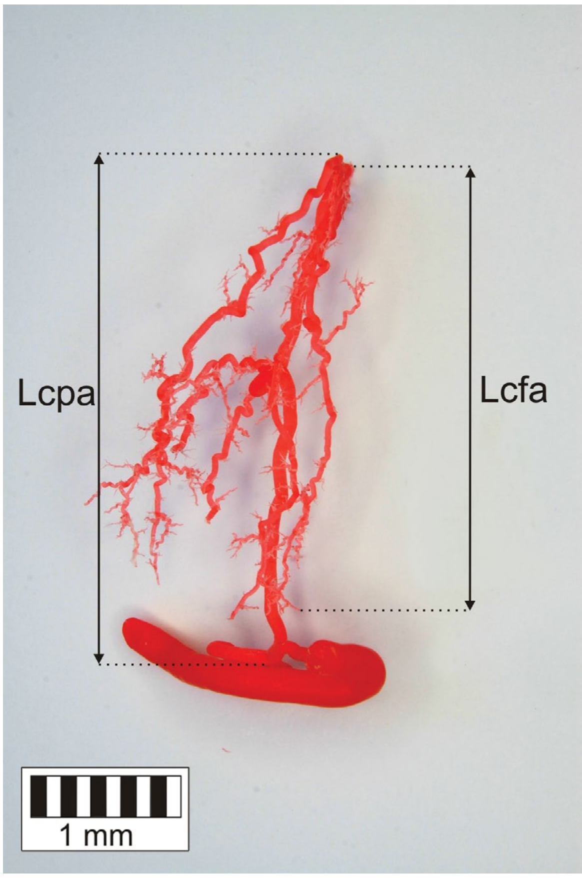

Figure 2. Corrosive cast of bovine intratesticular arteries (Plastogen G); Lcpa — maximal length of centripetal arteries; Lcfa — maximal length of centrifugal arteries.

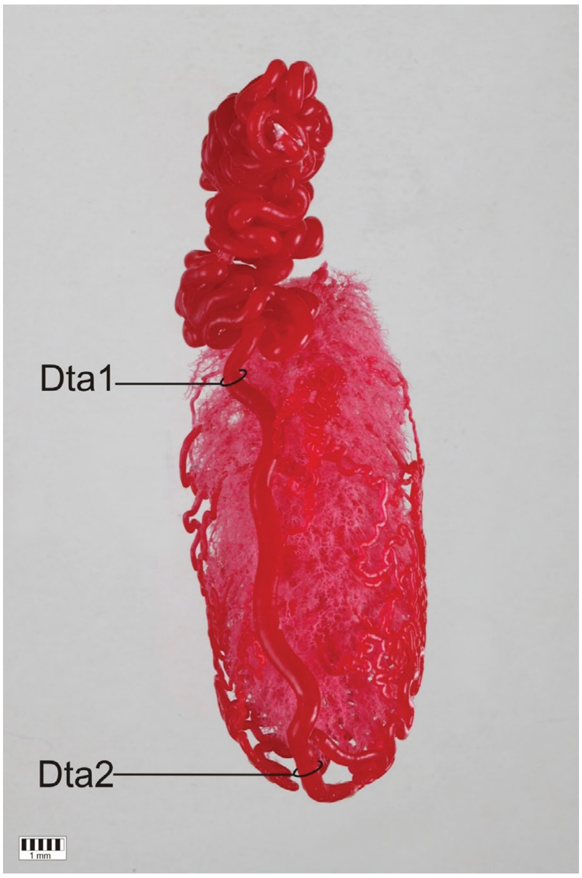

Figure 3. Corrosive cast of bovine arteries (Batson no. 17); Dta1 — diameter of testicular artery when leaving the spermatic cords on the posterior surface of the testis; Dta2 — diameter of testicular artery at the level of division on terminal branches.

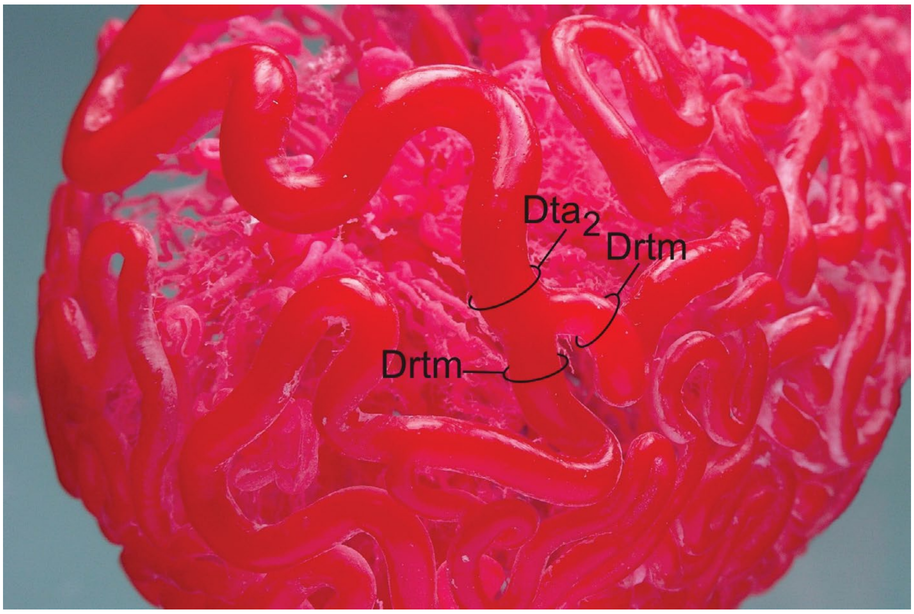

Figure 4. Corrosive cast of bovine arteries (Batson no. 17); Dta2 — the diameter of the testicular artery at the level of division on terminal branches; Drtm — the diameter of the rami tunicales majores after division into the testicular artery.

— Iq2 = diameter of testicular artery leaving the spermatic cords/maximal width of corrosion cast of testis (Dta1/Wta);
— Iq3 = diameter of testicular artery at the level of division into terminal branches/maximal width of corrosion cast of testis (Dta2/Wt);
— Iq4 = diameter of rami tunicales majores after division of testicular artery/maximal width of corrosion cast of testis (Drtm/Wt);
— Iq5 = maximal length of centrifugal arteries/maximal length of centripetal arteries (Lcfa/Lcpa).

Statistical analysis

The Iq indices were tested for normality with Shapiro-Wilk test. Following this, the results for the two groups of corrosion kits were compared using the Student's t-test for variables with a normal distribution, or the Mann-Whitney U test, for those with a non-normal distribution. Continuous variables with normal distribution are presented as means with standard deviation (SD), whereas those with a non-normal distribution are presented as medians with lower and upper quartile values (25%–75%). P-values lower than 0.05 were considered statistically significant. All analyses were performed using Statistica 13.1 (Dell) licensed to the Medical University of Lodz, Poland.

RESULTS

Qualitative assessment

Generally, the two groups of corrosion cast were found to be very similar qualitatively (Table 1). However, rami tunicales minores were found to be characterised by higher fragility in Plastogen G than Batson no. 17 (brittle vs. solid). In contrast, the rami tunicales minores demonstrated higher flexibility in Batson no. 17 than Plastogen G (flexible vs. stiff) (Table 1). Additionally, three casts from the Plastogen G group and one from the Batson no. 17 group demonstrated accessory branches arising from the testicular artery during its course from leaving the spermatic cords to the level of division at the terminal branches (Fig. 5).

Quantitative assessment

No significant differences were found between Plastogen G and Batson no. 17 casts for Iq1, Iq2, Iq4 or Iq5 (Table 3). However, Iq3 was significantly lower in the Plastogen G group (0.71 ± 0.01) than the Batson no. 17 group (0.79 ± 0.01; p = 0.0092 (Table 3, Fig. 6).

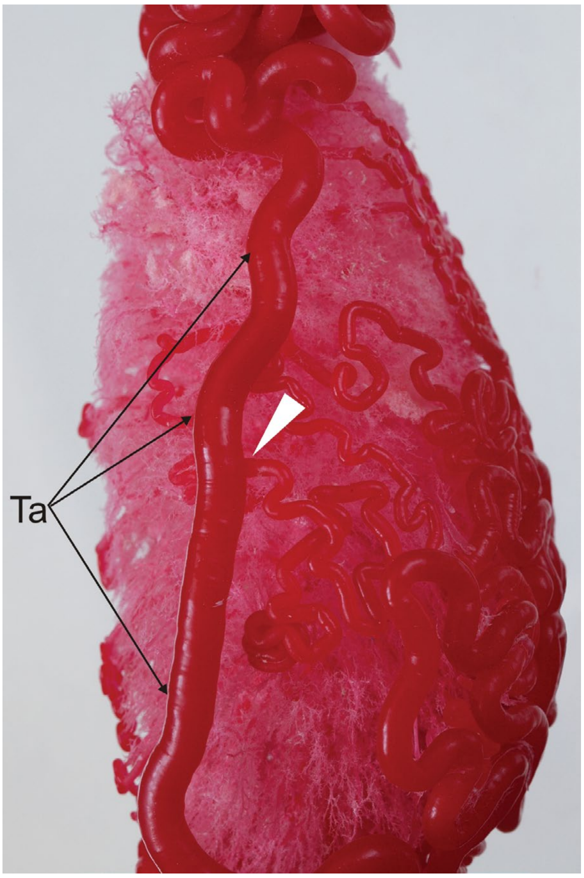

Figure 5. Corrosive cast of bovine arteries (Batson no. 17); Ta — testicular artery; arrowhead — accessory branch of testicular artery.

Table 3. Quantitative assessment of two groups of corrosion casts, comprising five indexes

Indexes	Plastogen G	Batson no. 17	P
Iq1	0.47 ± 0.04	0.47 ± 0.09	0.9977
Iq2	0.08 ± 0.01	0.07 ± 0.01	0.2484
Iq3	**0.07 ± 0.01**	**0.08 ± 0.01**	**0.0092**
Iq4	0.06 ± 0.01	0.06 ± 0.01	0.1968
Iq5	0.855 ± 0.0730.89 (0.80–0.90)	0.89 (0.79–0.90)	0.4148

Normally distributed data are presented as means with standard deviations. Non-normally distributed data are presented as medians with lower and upper quartile values

DISCUSSION

Injection or casting media require special physicochemical properties to allow the three-dimensional (3D) visualisation of vessels, their branches and anastomoses. Such properties include low viscosity, which allows them to pass through capillaries, as well as even and rapid polymerisation, minimal shrinkage during hardening, and resistance to corrosion, cleaning, dissection and drying procedures.

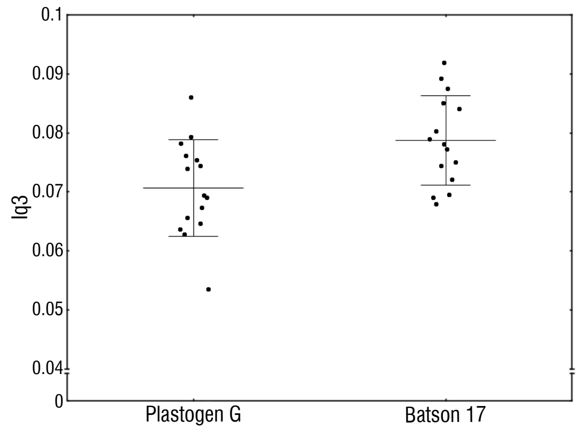

Figure 6. Comparison of Iq3 values between corrosion casts made using Plastogen G and Batson no. 17. Raw data and means with standard deviations are presented in the figure.

In addition, the medium should allow replication of endothelial structures [10, 12]. The choice of medium can greatly affect the results of any anatomical study. For example, while some authors have reported the length of the testicular artery in bulls to be within the 140–150 cm range [7], others estimated the same value to be within the 340–455 cm range [8], or even 700 cm [13].

Latex or silicone rubber compounds such as Cementex, Geon, Vultex, or Microfil have several disadvantages making them less suited to replicate the fine vascular microstructures, such as inconsistent replication of luminal surface structures, and lower resistance to corrosion and drying procedures [5, 12]. Latex materials are characterised by very good penetrability [1, 2, 12] and have a very small shrinkage ratio during polymerisation; however, most of the latex casts do not maintain their dimensional structure following treatment of the investigated tissues [6].

The two groups of corrosion casts in the present study were characterised by similar qualitative attributes; the only significant differences were that the rami tunicales minores in the Plastogen G group demonstrated greater fragility and lower flexibility than those in the Batson no. 17 group. Therefore, corrosive casts made using Batson no. 17 kit have a lower risk of suffering damage during transport or taking measurements.

The casting media can be injected into the blood vessels using a syringe or a syringe connected to a perfusion apparatus with a flow meter. It is important to adjust the injection pressure in order to fill the vascular bed. For instance, in the rat testis a resin perfusion ranging from 90 to 120 mmHg gives the best filling and cell replication [15, 16]. Good perfusion also depends on the organ. For example, a pressure of 80–100 mmHg may be used for hamster or rat heart, but a pressure of 15 mmHg would be more suitable for hamster lung [9, 10]. According to our previous studies [3, 14, 18, 19, 20–22] the casts used in the present study were injected into the blood vessels using a syringe without a perfusion apparatus equipped with a flow meter, as this is arguably unnecessary for obtaining good quality corrosion casts. Plastogen G effectively penetrates capillaries and small vessels, allowing assessments to be made of anastomoses in the spermatic cord [22], anastomoses in the tunica albuginea [20] and the types of intratesticular arteries [21]. In respect to testis such observation confirmed studies of other authors [11, 17, 23].

The quantitative analysis found the Iq3 index to be significantly lower in the Plastogen G group than the Batson no. 17 group. Iq3 was calculated as the ratio of the diameter of the testicular artery at the level of division into terminal branches to the maximal width of the corrosion cast of the testis. The maximal width of testis cast measured in transverse section (Wt) was approximately 15% higher in the Batson no. 17 group (49.18 mm) than the Plastogen G group (57.92 mm). However, the diameter of the testicular artery at the level of division in the Batson no. 17 group (4.53 mm) was approximately 24% higher than in the Plastogen G group (3.45 mm). Therefore, it appears that the difference in Iq3 can be attributed to the greater diameter of the testicular artery. This may be due to the fact that the frequency of the accessory branches of the testicular artery was three times higher in the Plastogen G group than the Batson no. 17 group. This arteries arise from the testicular artery during its course along the posterior surface of organ between the point where it leaves the spermatic cords and the level of division into terminal branches (Fig. 5). It is possible that in the Plastogen G group, i.e. in cases where the frequency of accessory branch of testicular artery is higher, the resin penetrated and influenced the lower diameter of the testicular artery at the level of its division.

A limitation of the study is fact that the testes were acquired from different populations of bulls. In addition no attempt was made to match morphological studies to age of the donor or position of the gonad (left or right).

CONCLUSIONS

Similar qualitative and quantitative attributes were found for the two tested groups of cast testis arterial vessels. Batson no. 17 appears to be a good resin for 3D visualisation of extra- and intratesticular arterial vessels.

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