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Testicular fixation and its effect on ipsilateral and contralateral testis in prepubertal rat model

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Abstract

Background: While performing orchidopexy, various suture materials or fibrin glues are used to achieve testicular fixation. This study was designed to assess the histological changes in testis after orchidopexy using fibrin glue and suture material.

Methods: Male Wistar rats ($n=80$) were divided randomly into four groups. Group I, ($n=20$): sham operation, Group II ($n=20$): Dartos Pouch (DP), Group III ($n=20$): Transtunical fixation (TF), Group IV ($n=20$): Tissue Adhesive (TA). Ipsilateral and contralateral testicular histology was assessed at 70 and 120 days of life after sacrificing animals by using thiopental sodium intraperitoneally at a dose of 100 mg/kg.

Results: Morphologically, at day 70, contralateral testis in Group III had a significant (p value 0.046) decrease testicular width (0.92 ± 0.01 vs 1.24 ± 0.39 cm). At 120 of life, Group I, II, III, and IV had a significant (p value < 0.001 each) decrease testicular width and weight in ipsilateral and decrease testicular length (p value 0.002) in contralateral testis. Histologically, mean seminiferous tubular diameter and DNA flow cytometry had a significant (p value < 0.001) decrease in size in Group I, II, III, and IV both ipsilateral as well as contralateral testis. Intergroup comparison at 70 and 120 days of life showed a significant decrease in seminiferous tubular diameter in Group II, III and IV and in Johnsen maturation score, seminiferous tubular diameter, DNA flow cytometry in Group I, II, III, and IV.

Conclusions: Dartos Pouch is most suitable procedure for treatment of orchidopexy. Suture fixation must be avoided and if the need arises then instead of suture materials, fibrin glue should be used for testicular fixation.

Keywords: Fibrin glue, Orchidopexy, Suture material

1 Background

Testicular fixation is of paramount importance in the setting of testicular torsion, and cases of the undescended testis, during orchidopexy. Testicular torsion is an emergency, occurring primarily in the preadult hood and young men entering in pubertal age. Prompt diagnosis and surgical intervention are essential to establish testicular blood flow and to avoid permanent damage to testis [1, 2].

It is hypothesized that intervention performed within 6 h of symptoms has more than 90% salvage rate, but the documented proof is not available. Historically, various surgical techniques are documented for orchidopexy [3–6]. But with the creation of dartos pouch, the pendulum has shifted towards the suture less orchidopexy. However, some surgeons still fix the testis even after placing them in dartos pouch, to prevent retraction at a later stage [7]. Recently, tissue adhesive is being used in place of suture material with a hypothesis that they are much safer and biologically adaptable [8]. Though few surgeons have tried fibrin glue for orchidopexy as well, yet the effect of fibrin glue on testicular parenchyma is not seen [9]. This study was designed to compare the effects of fibrin glue and suture material on testicular parenchyma in prepubertal and post-pubertal rats undergoing orchidopexy.

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1.1 Objectives

As testicular development in humans is akin to rats, it is assumed that the use of an experimental model in rats provides beneficial evidence for further research on the morphology and histology of the testis. The main objective of this experimental study was to study the morphological and histological changes caused by fibrin glue and suture material in testicular parenchyma of ipsilateral and contralateral testis in prepubertal rats.

2 Methods

2.1 Ethical statements

After obtaining clearance from the institute animal ethics committee vide reference no 726/IAEC/13 dated 14 March 2013, experimental procedures were carried out by following the guidelines for animal care as laid down by independent ethics committee protocol (www.iecindia.org).

2.2 Study design

Eighty-six male Wistar rats, aged 21 days, taken for the study from the animal house facility of the institute, were housed under standard laboratory conditions and allowed food and water ad libitum. On examination, scrotum found to be hypoplastic and testis felt in the groin region. Initially, first 6 rats were used to establish the standard operating procedure. Then, we randomly established 4 Groups: (I) sham operation ($n=20$); (II) dartos pouch ($n=20$); (III) Transtunical fixation ($n=20$); (IV) Tissue adhesive fixation ($n=20$).

2.3 Experimental procedures

All the procedures were performed in the animal laboratory after anaesthetizing the animals by using intraperitoneal ketamine (2 mg/kg body weight). All rats also received 50 mg/kg ceftriaxone intramuscularly during the preoperative period of the experiment. Under antiseptic precautions, rat was positioned on the board, its ventral surface shaved and prepared with povidone iodine. The standard procedure consists of longitudinal incision in the lower abdomen followed by identification and mobilization of the testis. Rest procedures were performed according to the allotted group. In Group I, incision is given and closed without doing anything. Group II dartos pouch created by giving transverse scrotal incision to exposed underlying dartos layer. The dartos and tunica vaginalis layer were sharply incised, and the testis was placed in the dartos pouch. Group III testis was suture to the tunica vaginalis using Prolene 5-0 near both upper and the lower pole of the testis. Group IV testis was fixed to the tunica vaginalis using tissue glue (N-Butyl 2-cyanoacrylate Glubran 2). All the rats were operated by the single surgeon and on the left side only. Contralateral right testis remained untouched and was used for comparison with the left testis. Postoperatively rats were returned to their respective cages. They were provided with an equal amount of commercial feed twice on a schedule time every day. Water was made available ad libitum. Ten rats from each group were sacrificed at 70 days of life and the remaining 10 rats in each group at 120 days of life, by using thiopental sodium intraperitoneally at a dose of 100 mg/kg. Both ipsilateral and contralateral testes were harvested and divided into two halves. One hemi testis was fixed in Bouin's solution for 24 h and processed for histological examination to evaluate the Johnsen score. Thick sections were cut transversely (5 μm) and stained with haematoxylin and eosin (H & E) and periodic acid-Schiff. Inflammatory changes were graded according to the system proposed by Dixon et al. [10]. In this system, 0 = no abnormality; 1 = mild lesion affecting 5% of the area; 2 = moderate lesion affecting 25–75% of the area; and 3 = severe lesion affecting 75% of the area. Using a 25× objective, 25 tubules in one section of the biopsy were evaluated and scored for assessment of the degree of testicular maturity according to the criteria [11]. For estimation of seminiferous tubular diameter (STD): in every H & E section, a minimum of 25 circular tubules were measured in two axes drawn perpendicular to each other using a computerized image-analysis system with a Pentium processor-based computer (Celebris XL, Digital Corp, USA), an Olympus research microscope (Olympus, USA), a 10-bit digital camera (Correco, Canada), and Optimas 5.2 image analysis software (Optimas, USA) as described by us earlier [12]. The other hemi testis was transported in Roswell Park Memorial Institute solution for DNA flow cytometry. For, DNA Flow cytometry, testes were separated from the tunica albuginea and minced in phosphate saline (PBS). The resultant single-cell suspension was washed twice in PBS, and a 100ul aliquot of the suspension was fixed in 70% ethanol in PBS. After centrifugation, the pellets were resuspended in propidium Iodine and subjected to RNA digestion by enzyme RNase (Sigma USA). DNA histogram was obtained on a flow cytometer (Becton–Dickinson FAC scan, USA). The data thus obtained were analysed by Cell Fit software.

Experimental animals: Prepubertal male Wistar rats, aged 21 days.

Housing and husbandry: Animals were procured from the institute animal laboratory and were caged in the laboratory itself. They were allowed food and water ad libitum.

Sample size: Eighty prepubertal Wistar rats were divided into IV Groups where each group contains 20 rats. Control group; 20 rats, interventional group; 60 rats.

Allocating animals to experimental groups: All rats belong to same species and comparable in terms of age and weight. Random allocation of group was done using block randomization technique. First, all the 80 rats were given serial number between 1 and 80. Block randomization technique was used to allocate the rats between two groups (Ipsilateral testis, Contralateral testis for each 40 rats). Further block randomization technique was used to allocate the 40 rats into 4 groups with 10 rats in each of the four groups for ipsilateral testis and contralateral testis groups.

2.4 Statistical methods

The normality of the variables was assessed using Shapiro–Wilk test. Continuous variables were presented in mean ± standard deviation. Independent samples *t* test was used to compare the means between two independent groups. One-way ANOVA test was used to compare the means between four study groups (Group I, II, III, & IV). When one-way ANOVA test was significant, multiple comparisons were performed using Bonferroni corrections. *p* Value < 0.05 was considered as statistically significant. Statistical package for social sciences version-23 (SPSS-23, IBM, Chicago, USA) was used for data analysis.

3 Results

All animals survived the time of the experimental study. Data collected include testis size (length, width), testis weight, degree of testicular parenchymal inflammatory changes, seminiferous tubular diameter, Johnsen score, and DNA flow cytometry scores of ipsilateral and contralateral testes at 70 and 120 days of life.

3.1 Testicular size and weight

Table 1 shows the mean weight and size of ipsilateral as well as contralateral testis of all groups at 70 days of life. The result showed that the mean difference between four study groups was found to be statistically insignificant for mean length, width and weight of the ipsilateral testis as well as contralateral testis (***p* > 0.05 each**). On comparison, between ipsilateral and contralateral testis at 70 days of life, group III showed a significant decrease in testicular size in width ($p < 0.05$). There was no significant difference in testicular weight or size in rest. When the testis was compared at 120 days of life, the mean difference between four study groups was found to be statistically significant for mean width and weight of the ipsilateral testis and mean length for contralateral testis (***p* < 0.05 each**). In between two testes, (ipsilateral and contralateral testis), the mean difference was significant for the width of the contralateral testis in group III ($p < 0.05$) and for the rest of others, the mean difference was statistically insignificant ($p > 0.05$) (Table 2).

3.2 Testicular histology

The group I showed no inflammation either at 70 or 120 days of life (Fig. 1). Mild inflammation of testicular parenchyma in the ipsilateral testis was noted in group III after 70 days of life (Fig. 1b). Similarly, mild inflammation was found in group IV after 70 days of life. Suture material reaction was present in the group III after 120 of life (Fig. 1c). We observed neither necrosis nor severe inflammation in any groups, either in ipsilateral or in contralateral testis after 70 or 120 days of life.

Table 1 Mean testicular size and weight at 70 days of life ($n = 80$)

Group	Ipsilateral testis ($n = 40$)			Contralateral testis ($n = 40$)		
	Length (cm)	Width (cm)	Weight (gm)	Length (cm)	Width (cm)	Weight (gm)
I ($n = 10$)	2.11 ± 0.49	1.26 ± 0.78	1.329 ± 0.69	2.10 ± 0.98 [0.977]	1.24 ± 0.39 [0.943]	1.319 ± 0.91 [0.978]
II ($n = 10$)	2.09 ± 0.53	1.24 ± 0.37	1.317 ± 0.65	2.07 ± 0.66 [0.941]	1.23 ± 0.88 [0.974]	1.304 ± 0.89 [0.955]
III ($n = 10$)	1.76 ± 01.04	0.78 ± 1.24	1.004 ± 0.84	1.98 ± 0.81 [0.879]	0.92 ± 0.01 **[0.046]**	1.295 ± 1.05 [0.824]
IV ($n = 10$)	1.96 ± 0.21	1.17 ± 0.43	1.216 ± 0.59	1.82 ± 1.23 [0.983]	0.84 ± 0.23 [0.924]	1.248 ± 1.02 [0.932]
p Value	0.601	0.491	0.715	0.911	0.173	0.998
PHT ($p < 0.05$)	NA	NA	NA	NA	NA	NA

Data presented in mean ± standard deviation. One-way ANOVA test used between four (I, II, III, IV) groups followed by Post hoc test.; Independent samples *t* test used between ipsilateral hemi testis and contralateral hemi testis. PHT = Post hoc test, NA = Not Applicable. ***p* < 0.05 significant**

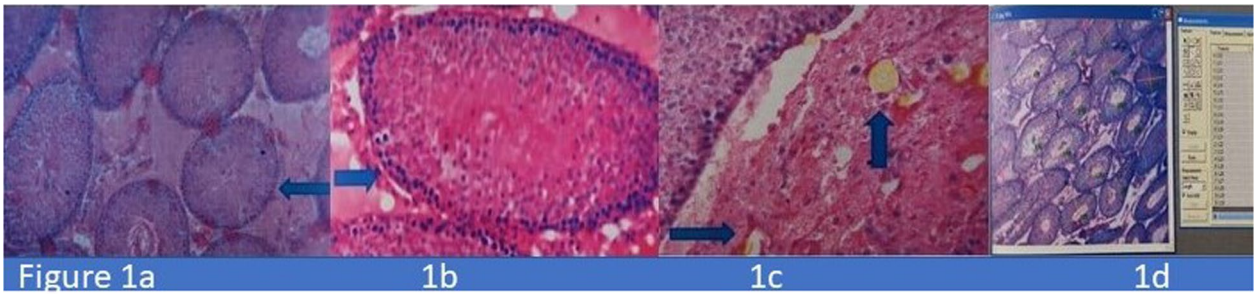

Fig. 1 **a** Normal testicular parenchyma in Group I. **b** Mild inflammation in Group III at 70 days of life. **c** Inflammation around suture material in Group III at 120 days of life. **d** Seminiferous tubular diameter in Group I at 70 days of life

Table 2 Mean testicular size and weight at 120 days of life ($n=80$)

Group	Ipsilateral testis ($n=40$)			Contralateral testis ($n=40$)		
	Length (cm)	Width (cm)	Weight (gm)	Length (cm)	Width (cm)	Weight (gm)
I ($n=10$)	2.28 ± 0.84	1.29 ± 0.36	1.392 ± 0.62	2.23 ± 0.06 [0.853]	1.37 ± 0.33 [0.601]	1.481 ± 0.41 [0.706]
II ($n=10$)	2.49 ± 0.39	1.20 ± 0.51	1.391 ± 0.67	2.51 ± 0.51 [0.923]	1.32 ± 0.71 [0.669]	1.428 ± 0.68 [0.896]
III ($n=10$)	1.78 ± 0.98	0.52 ± 0.02	0.764 ± 0.03	2.01 ± 0.04 [0.468]	1.01 ± 0.06 **[0.001]**	0.916 ± 0.31 [0.140]
IV ($n=10$)	1.89 ± 0.37	1.02 ± 0.48	1.281 ± 0.42	1.96 ± 0.4 9[0.723]	0.94 ± 0.21 [0.635]	1.187 ± 0.93 [0.784]
p Value	0.099	**0.001**	**0.024**	**0.002**	0.052	0.200
PHT ($p<0.05$)	NA	1–3, 2–3, 3–4	2–3	2–3, 4	NA	NA

Data presented in mean ± standard deviation. One-way ANOVA test used between four (I, II, III, IV) groups followed by Post hoc test.; Independent samples *t* test used between ipsilateral hemi testis and contralateral hemi testis. PHT = Post hoc test, NA = Not Applicable. ***p* < 0.05 significant**

3.3 Johnsen maturation score

At 70 days of life, in ipsilateral testis, there was no significant difference between the groups I (9.24 ± 0.12), II (9.28 ± 0.18), III (9.28 ± 0.21) and IV (9.24 ± 0.16) and in the contralateral testis groups I (9.27 ± 0.12, *p* value 0.583), II (9.38 ± 0.26, *p* value 0.311), III (9.23 ± 0.16, *p* value 0.557), group IV (9.25 ± 0.14, *p* value 0.883) (Table 3).

At 120 days of life, in ipsilateral testis, there was a significant difference between the groups I (9.09 ± 0.28), II (9.11 ± 0.23), III (8.73 ± 0.23), and IV (9.32 ± 0.31) and in the contralateral testis, group III (8.77 ± 0.18, *p* value < 0.001), and IV (9.12 ± 0.06, *p* value 0.001) showed a significant difference (Table 4).

Intergroup comparison of the ipsilateral testis of 70 and 120 days of life showed a significant difference in group III (8.73 ± 0.23, *p* value 0.001) only (Table 5), while on contralateral side, group I (8.90 ± 0.31, *p* value 0.002) III (8.77 ± 0.18, *p* value 0.001) and IV (9.12 ± 0.06, *p* value 0.015) showed a significant difference (Table 6).

3.4 Seminiferous tubular diameter

At 70 days of life, in ipsilateral testis, there was a significant difference between the groups I (264 ± 4.01 cm), II (246 ± 1.37 cm), III (207 ± 0.46 cm) and IV (238 ± 1.59 cm) and in the contralateral testis groups III (220 ± 12.24 cm, *p* value 0.003), and IV (228 ± 1.77 cm, *p* value 0.001) showed significant differences (Table 3; Fig. 1d). As compared to group I, group IV showed a significant decrease in tubular diameter. At 120 days of life, in ipsilateral testis, there was significant difference between the groups I, II, III, and IV (*p* value < 0.001) and in the contralateral testis groups III (220 ± 12.30 cm, *p* value < 0.020), IV (228 ± 1.66 cm, *p* value 0.001) showed a significant difference (Table 4). Intergroup comparison of ipsilateral testis at 70 and 120 days of life showed a significant difference in the groups I, II, III and IV (*p* value 0.001) (Table 5), while on contralateral side, group I (260 ± 0.82 cm, *p* value 0.006), III (209 ± 6.00 cm, *p* value 0.001) and IV (242 ± 2.43 cm, *p* value 0.004) showed a significant difference (Table 6) signifying that intervention

Table 3 Mean Johnson score, Seminiferous tubular diameter and DNA Flow cytometry at 70 days of life ($n=80$)

Group	Ipsilateral testis ($n=40$)			Contralateral testis ($n=40$)		
	JS	STD	DFC	JS	STD	DFC
I ($n=10$)	9.24 ± 0.12	264 ± 4.01	63.25 ± 1.70	9.27 ± 0.12 [0.583]	262 ± 4.04 [0.281]	60.50 ± 1.29 **[0.007]**
II ($n=10$)	9.28 ± 0.18	246 ± 1.37	60.00 ± 0.81	9.38 ± 0.26 [0.331]	234.42 ± 1.96 [0.251]	60.25 ± 0.50 [0.417]
III ($n=10$)	9.28 ± 0.21	207 ± 0.46	61.25 ± 0.95	9.23 ± 0.16 [0.557]	220 ± 12.24 **[0.003]**	64.25 ± 2.50 **[0.002]**
IV ($n=10$)	9.24 ± 0.16	238 ± 1.59	60.75 ± 0.95	9.25 ± 0.14 [0.883]	228 ± 1.77 **[0.001]**	64.50 ± 1.29 **[0.001]**
p Value	0.907	**< 0.001**	**< 0.001**	0.255	**< 0.001**	**< 0.001**
PHT ($p<0.05$)	NA	1–2, 2–4	1–2, 3, 4	NA	1–2, 3, 4 & 2–3 & 3–4	1–3, 4 & 2–3

Data presented in mean ± standard deviation. One-way ANOVA test used between four (I, II, III, IV) groups followed by Post hoc test.; Independent samples *t* test used between ipsilateral hemi testis and contralateral hemi testis. PHT = Post hoc test, NA = Not Applicable. ***p* < 0.05 significant**

JS = Johnsen Score, STD = seminiferous Tubular diameter, DFC = DNA Flow cytometry

Table 4 Mean Johnson score, Seminiferous tubular diameter, and DNA Flow cytometry at 120 days of life ($n=80$)

Group	Ipsilateral testis ($n=40$)			Contralateral testis ($n=40$)		
	JS	STD	DFC	JS	STD	DFC
I ($n=10$)	9.09 ± 0.28	260 ± 0.82	63.25 ± 1.70	8.90 ± 0.31 [0.168]	259 ± 2.03 [0.166]	60.50 ± 1.29 **[0.009]**
II ($n=10$)	9.11 ± 0.23	239 ± 2.46	62.00 ± 0.81	9.32 ± 0.14 [0.236]	238 ± 2.27 [0.367]	62.00 ± 1.63 [0.999]
III ($n=10$)	8.73 ± 0.23	209 ± 6.00	61.25 ± 0.95	8.77 ± 0.18 **[0.001]**	220 ± 12.30 **[0.020]**	64.25 ± 2.50 **[0.002]**
IV ($n=10$)	9.32 ± 0.31	242 ± 2.43	60.75 ± 0.95	9.12 ± 0.06 [0.060]	228 ± 1.66 **[0.001]**	64.50 ± 1.29 **[0.001]**
p Value	**0.002**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
PHT ($p<0.05$)	1–3, 2–3, 3–4	1–2, 4 & 2–3, 3–4	1–2, 3, 4 & 2–4	1–2, 2–3 & 3–4	All	1–4, 2–3, 4

Data presented in mean ± standard deviation. One-way ANOVA test used between four (I, II, III, IV) groups followed by Post hoc test.; Independent samples *t* test used between ipsilateral hemi testis and contralateral hemi testis. PHT = Post hoc test, NA = Not Applicable. ***p* < 0.05 significant**

*JS = Johnsen Score, STD = seminiferous Tubular diameter, DFC = DNA Flow cytometry

in ipsilateral testis also had its effect on the contralateral testis as well.

3.5 DNA flow cytometry (DFC)

At 70 days of life, in ipsilateral testis, there was a significant difference between the groups I, II, III, and IV (p value < 0.001) and in contralateral testis group I (60.50 ± 1.29, p value 0.007), III (64.25 ± 2.50, p value 0.002) and IV (64.50 ± 1.29, p value 0.001) showed significant differences (Table 3). At 120 days of life, in the ipsilateral testis, there was a significant difference between the groups I, II, III, and IV (p value < 0.001) and in contralateral testis group I (64.50 ± 1.29, p value < 0.009), III (64.25 ± 2.50, p value 0.002) and IV (64.50 ± 1.29, p value 0.001) showed a significant difference (Table 4). Intergroup comparison of ipsilateral testis of 70 and 120 days of life showed a significant difference in the groups I, II, III and IV (p value 0.002) (Table 5), similarly on contralateral side significant difference (p value 0.001) was found in the groups I, II, III, IV (Table 6).

3.6 DNA histogram

Significant reduction in haploid cell population in group III, and IV as compared to I. On intergroup analysis, only group III showed significant difference in haploid cell population (Fig. 2a, b).

Table 5 Comparison between ipsilateral testis at 70 and 120 days of life of in four study groups

Groups	Ipsilateral testis 70 days of life ($n=40$)			Ipsilateral testis 120 days of life ($n=40$)		
	JS	STD	DFC	JS	STD	DFC
I ($n=10$)	9.24 ± 0.12	264 ± 4.01	63.25 ± 1.70	9.09 ± 0.28 [0.137]	260 ± 0.82 **[0.006]**	63.25 ± 1.70 [0.999]
II ($n=10$)	9.28 ± 0.18	246 ± 1.37	60.00 ± 0.81	9.11 ± 0.23 [0.511**]**	239 ± 2.46 [0.307]	62.00 ± 0.81 [0.258]
III ($n=10$)	9.28 ± 0.21	207 ± 0.46	61.25 ± 0.95	8.73 ± 0.23 **[0.001]**	209 ± 6.00 **[0.001]**	61.25 ± 0.95 [0.999]
IV ($n=10$)	9.24 ± 0.16	238 ± 1.59	60.75 ± 0.95	9.32 ± 0.31 [0.488]	242 ± 2.43 **[0.004]**	60.75 ± 0.95 [0.999]
p Value	0.907	**< 0.001**	**< 0.001**	**0.002**	**< 0.001**	**0.002**
PHT ($p<0.05$)	NA	1–2, 2–4	2–3, 4 & 3–4	2–3 & 3–4	1–2, 4 & 2–3, 3–4	1–2, 3, 4 & 2–4

Data presented in mean ± standard deviation. One-way ANOVA test used between four (I, II, III, IV) groups followed by Post hoc test.; Independent samples *t* test used between ipsilateral hemi testis measured at 70 day and 120 days of life. PHT = Post hoc test, NA = Not Applicable. ***p* < 0.05 significant**

*JS = Johnsen Score, STD = seminiferous Tubular diameter, DFC = DNA Flow cytometry

Table 6 Comparison between Contralateral testis at 70 and 120 days of life in four study groups ($n=80$)

Groups	Contralateral testis 70 days of life ($n=40$)			Contralateral testis 120 days of life ($n=40$)		
	JS	STD	DFC	JS	STD	DFC
I ($n=10$)	9.27 ± 0.12	262 ± 4.04	60.50 ± 1.29	8.90 ± 0.31 **[0.002]**	259 ± 2.03 **[0.049]**	60.50 ± 1.29 [0.999]
II ($n=10$)	9.38 ± 0.26	234.42 ± 1.96	60.25 ± 0.50	9.32 ± 0.14 [0.528]	238 ± 2.27 [0.3155**]**	62.00 ± 1.63 [0.219**]**
III ($n=10$)	9.23 ± 0.16	220 ± 12.24	64.25 ± 2.50	8.77 ± 0.18 **[0.001]**	220 ± 12.30 **[0.001**]	64.25 ± 2.50 [0.999]
IV ($n=10$)	9.25 ± 0.14	228 ± 1.77	64.50 ± 1.29	9.12 ± 0.06 **[0.015]**	228 ± 1.66 [0.999]	64.50 ± 1.29 [0.999]
p Value	0.255	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
PHT ($p<0.05$)		1–2, 3, 4 & 2–3 & 3–4	1–3, 4 & 2–3, 4	1–3, 4 & 2–4	All	1–2, 3–4

Data presented in mean ± standard deviation. One-way ANOVA test used between four (I, II, III, IV) groups followed by Post hoc test.; Independent samples *t* test used between Contralateral hemi testis measured at 70 day and 120 days of life. PHT = Post hoc test, NA = Not Applicable. ***p* < 0.05 significant**

*JS = Johnsen Score, STD = seminiferous Tubular diameter, DFC = DNA Flow cytometry

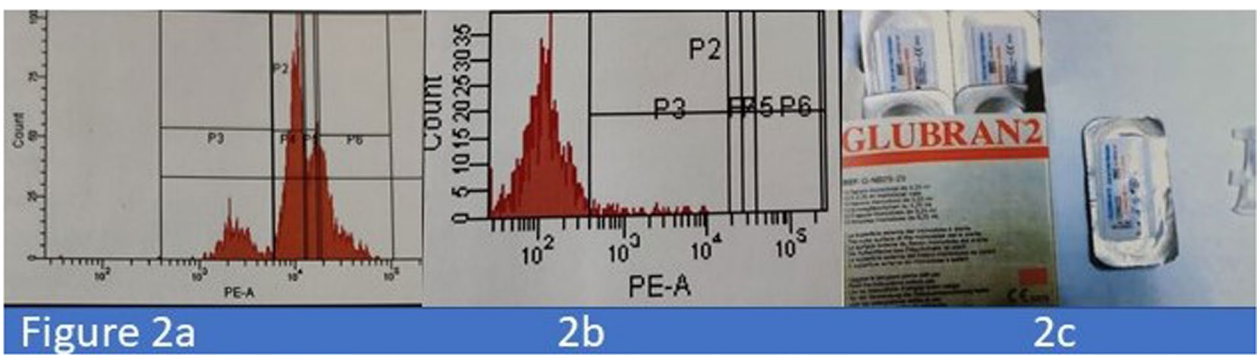

Fig. 2 **a** Group I haploid cell population percentage. **b** Group III haploid cell population percentage. **c** Tissue Adhesive Glubran 2

4 Discussion

There is considerable variation in surgical practice of operative techniques used for orchidopexy. The literature on orchidopexy technique is heterogeneous and scarce, with variation reported in the type of suture use, fixation methods, synchronous procedures, and contralateral testicular fixation [13]. The fixation of the testis by whatever methods used has some degree of a detrimental effect on the spermatogenesis of the testis. The landmark animal experiment done by Bellinger et al. in 1989 showed the detrimental effects of suture material on testicular parenchyma [14]. Recently I. Surer et al., in an experimental study, conducted on 42 prepubertal rats emphasized the detrimental effects of suture fixation on testicular parenchyma. They found an increase in the levels of the final products of lipid peroxidation and free radicals, as well as an increase in the consumption of antioxidant reserve systems, in both operated and contralateral testes. They concluded that the routine uses of testicular parenchymal sutures fixation should be avoided [15]. As noted previously, in another experimental model, Ribeiro et al. demonstrated that suture testicular fixation resulted in adverse histological consequences [16]. Dixon et al. advocated the technique of suture less dartos pouch orchidopexy, which is presently being practiced worldwide by most surgeons [10]. Recently, Noske HD reported the use of fibrin glue for fixation in 100 patients of extra vaginal torsion. They reported that there was no recurrence in these 100 cases [9]. Villet R et al., in a study on 40 female rats undergoing partial ovariotomy, assessed the effects of fibrin glue and suture material and observed that atrophic and fibrotic changes were minimal in fibrin glue group as compared to suture group [17]. Sencan A et al. studied the effect of fibrin glue as compared to transparenchymal suture fixation in 28 Westar rats [18]. In their study, only the testicular inflammation and seminiferous tubular diameter were assessed. The fibrin glue used was (Beriplast P 1 ml comby-set, Farma-tek Drug Industry, Istanbul, Turkey). They concluded that fibrin glue causes less tissue damage as compared with silk or polypropylene sutures. Our study was planned with the aim to compare the changes in the morphology and histology of testicular parenchymal tissue after the use of modern tissue adhesive, monofilament suture material, and surgical technique of dartos pouch. Tissue adhesive used for our study was GLUBRAN 2 Isobutyl-2-cyanoacrylate (GEM Srl, Viareggio, Italy) (Fig. 2c). It is a synthetic biodegradable cyanoacrylate basis glue, Class III CE product. It has high adhesive and haemostatic properties. It forms strong connections and rapidly polymerizes when the tissues is in contact with water and blood like environments. In medicine today, cyanoacrylates are being widely used as embolic material in various surgical procedures [19, 20].

In this experimental study, suture material Prolene 5-0 was used purposefully after review of literature showed that monofilament causes least tissue reaction among the various sutures [10]. This study shows that the morphological changes were significantly higher in the suture material Group III as compared to IV, II or I Group. Surprisingly, the contralateral testis also showed morphological changes after 120 days of life not only in group III but also in the group IV as well. This highlighted the facts that orchidopexy either by using transparenchymal suture fixation or using tissue adhesive material has element of testicular damage to a large extent. Recently published meta-analysis by Anand s et al. also advocated orchidopexy without transparenchymal suture fixation [21]. Similarly, antigenic and cytotoxic property of Glubran 2 cannot be ruled out completely. A Ayyildiz et al. studied the effects of cyanoacrylate on rat testis and urethra and observed damage to the seminiferous tubules, decreased spermatogenesis and tunica albuginea irregularity [22]. Cylwik et al. also highlighted the adverse effect after embolization of dog kidneys with cyanoacrylate [23].

The current study supports the experimental data of Rodriguez and Kaplan that the dartos Pouch fixation is the safest method of doing orchidopexy [7]. Similar observation was reported by Dixon et al. in their study on 120 prepubertal rats using various suture material and suture less dartos pouch [10].

We observed degenerative changes in both the testis at 70 and 120 days of life after use of suture and tissue adhesive. Though a long-term follow-up study is warranted to assess the effect of these testicular changes on the rate of fertility, this experimental study strongly highlighted the point that the use of either suture material or tissue adhesive is associated with testicular parenchymal injury and therefore, both should not be used for fixation.

5 Conclusions

Based on the findings of this study, we are of the opinion that the suture less dartos pouch orchidopexy is the best available option available in the present scenario. In cases of the testis requiring fixation, fibrin glue should be used instead of any suture material.

Abbreviations

Group I; SO: Sham Operation; Group II; DP: Dartos Pouch; Group III; TF: Tunica Transtunical fixation; Group IV; TA: Tissue Adhesive; H &E: Haematoxylin and eosin; STD: Seminiferous tubular diameter; JS: Johnsen maturation score; DFC: DNA Flow cytometry.

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Authors' contributions
AS had designed the study, done the surgical procedures, drafted the manuscript. MS had done the process of data analysis, critical review and revision of manuscript. SM done the histopathological analysis of study samples. All authors have read and approved the manuscript.

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Declarations

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Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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