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The influence of fixation on the cardiac tissue in a 1-year observation of swine hearts

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Abstract

Aim: The aim of this study was to investigate the influence of formaldehyde-based fixation on dimension, weight and shape of cardiac tissue during a 1-year observation.

Materials and methods: Seven measuring sites were permanently marked in 40 swine hearts prior to fixation. Four study groups (n = 10 each) were assembled that differed only in concentration and the type of fixative. The fixatives were 2%, 4% or 10% formaldehyde phosphate-buffered solution (FPBS) and alcoholic formalin. The samples were measured before fixation and then after fixation at 1 week, 3, 6 and 12 months.

Results: At the 3-month point, the 10% FPBS had caused significant changes in the smallest number of parameters, while the 2% FPBS affected the greatest number of dimensions. The most significant changes included chordae tendineae shrinkage and an increase in muscle thickness. After 6 months, the most significant changes were observed in 2% and 4% FPBSs and were also mainly associated with an increase in muscle thickness and chordae tendineae shrinkage. 1-year preservation compared to the baseline showed the most significant changes in muscle tissue thickness and hearth weight. The artery diameter decreased in long-term fixation in every tested solution. For atrial and angle measurements, 4% FPBS caused most significant changes among investigated fixatives.

Conclusions: In all tested solutions, long-term fixation significantly changed cardiac tissue dimension compared to the nonpreserved samples. Short-term to 1-year fixation changes are smaller, but they should not be neglected. Different fixatives should be used depending on the character of the planned measurements.

KEYWORDS

alcoholic formalin, formalin, long-term fixation, tissue preservation

1 | INTRODUCTION

Alexander Butlerov synthesized formaldehyde in 1859, more than 150 years ago.A few years later, Ferdinand Blum, a German biologist and physician, discovered the antiseptic and fixing ability of formaldehyde (Fox, Johnson, Whiting, & Roller, 1985). Since then, the role of formaldehyde in fixation has significantly grown and is now indisputable. Fixation is cheap, relatively simple and widely used in biological sciences including medicine (Thavarajah, Mudimbaimannar, Rao, Ranganathan, & Elizabeth, 2012). Despite this, only a few

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research studies have examined the influence of fixation on tissue shape and dimension (Hołda, Klimek-Piotrowska, Koziej, Piątek, & Hołda, 2016; Hołda, Klimek-Piotrowska, Koziej, Tyrak, & Hołda, 2017; Jonmarker, Valdman, Lindberg, Hellström, & Egevad, 2006; Vent et al., 2014). Nevertheless, long-lasting formaldehyde-based fixation is widely used, not only for specimens used for educational purposes but also for morphometric studies. Yet, there is still no information about tissue size changes during fixation over a long period of observation. Lack of such data is a main limitation of cardiac morphometric studies. On the whole, these studies are carried out on fixed samples. Therefore, the practical usefulness of those studies may be disputed. Moreover, the most effective type of fixation solution, which results in the smallest changes in tissue dimensions, remains unknown. At last, tissue shrinkage often takes place in biological tissues during the different phases of preparation for microscopical evaluation and that can have detrimental effects on the stereological estimates (Dorph-Petersen, Nyengaard, & Gundersen, 2001; West, 2013).

Thus, the aim of this study was to investigate the influence of different types and concentrations of formaldehyde-based reagents on the dimension, weight and shape of large cardiac tissue samples over 1-year period of observation.

2 | MATERIALS AND METHODS

This study was conducted on 40 swine hearts (*Sus scrofa f. domestica*). The authors want to emphasize, that all samples were intended for use in the food industry, so no animals have suffered because of this study. The slaughter was performed according to current reference standards (Council Regulation (EC) No. 1099/2009) (European Commission, 2009). In brief, animals were electrically stunned by head tongs with high frequency, but low voltage (60–80 V) electricity to produce unconsciousness. After stunning pigs were bleeded in horizontal position by the deep incision in the centre of the neck to cut carotid arteries and jugular veins (Grandin, 2013). The hearts were dissected in the routine manner form the thorax cavity within one hour after commercial slaughter of the animals. After removal of the hearts, all samples were washed in saline solution to get rid of blood and blood clothes. Hearts were dissected by an incision applied, starting from the apex of the heart, near the interventricular septum along the long-axis of the heart. The right and left atrium were also opened by the same incision (Hołda et al., 2016).

As described before by Hołda et al. (2016), the following measurement points were permanently marked in every heart, using pins and sutures: the thickness of the left ventricle free wall (halfway between apex and left atrioventricular ring), the thickness of the right ventricle free wall (in the middle), the diameter of the papillary muscle inside the left ventricle at its base, the length of the chordae tendineae (strut cord, connected to the anterior leaflet of left atrioventricular valve), the inner diameter of the left anterior descending artery, branch of the left coronary artery, the length marked between two pins on the interatrial septum surface, and the angle between three pins on the epicardial surface of the left ventricle (to investigate the degree of tissue rotation). After marking all described points, samples were weighed and measured prior to fixation.

The hearts were randomly assigned to one of the following study groups, differing only in terms of fixative type and concentration: (a) 2% formaldehyde phosphate-buffered solution (FPBS); (b) 4% FPBS; (c) 10% FPBS; and (d) alcoholic formalin solution (Alc; 85.5% absolute ethanol, 3.8% formaldehyde, 0.05% calcium acetate in distilled water). Next, the samples were immediately immersed in one of the fixatives. All the samples were stored individually at room temperature (21°C) in closed containers (fixative to tissue ratio = 40:1) (Hołda et al., 2016).

Next, heart samples were repeatedly weighted and measured at the same marked points at one week, three months, six months and 12 months following fixation. We performed linear measurements using 0.03 mm precision electronic callipers (YATO YT–7201, Poland) and angle measurements using a 1-degree precision half-circle protractor. At all time, the measurements of all marked structures were performed by two independent researchers to minimize human bias. When a difference between two measurements exceeded 5%, the measurements were repeated. The mean of two measurements was calculated.

Data were presented as median values with corresponding lower and upper quartiles as well as relative (percentage) changes. Friedman's nonparametric test was implemented to evaluate whether parameters changed significantly over time in a certain group. If the Friedman's test results were statistically significant, we performed a post hoc analysis to assess differences in value between different time points. The ANOVA or the Kruskal–Wallis test with post hoc analysis was used to compare the differences in specimen change between baseline measurements prior to fixation and one week, three months, six months and 12 months following fixation for each solution in a particular parameter. Statistical analyses were conducted using STATISTICA v13.1 (StatSoft, Inc., Tulsa, OK, USA). A p-value <0.05 was considered to be statistically significant.

3 | RESULTS

Table 1 shows the median values of the heart weight and the dimensions of the measured structures before and after fixation at the designated time points in different types and concentrations of reagents. Table 2 and Figure 1 present percentage changes of previously mentioned measurements during the fixation process.

3.1 | Baseline—3-month changes

After 3 months of fixation in a 2% FPBS a significant increases of muscle tissue thickness (papillary muscle diameter: +32.3%, $p < 0.05$; left ventricle thickness: +12.7%, $p < 0.05$; right ventricle thickness: +33.0%, $p < 0.05$) and contraction of chordae tendineae (–11.9%,

TABLE 1 Median values of measured heart parameters before fixation and at consecutive time points for samples preserved in different fixatives

Parameter	Study group	Before fixation	1 week	3 month	6 month	12 month	
		Me (Q1; Q3)	Me (Q1; Q3)	Me (Q1; Q3)	Me (Q1; Q3)	Me (Q1; Q3)	p^a
Heart weight (g)	2% FPBS (*n* = 10)	223.5 (209.0; 247.0)	233.0[b] (217.0; 257.0)	226.0 (209.0; 248.0)	228.0 (212.0; 250.0)	225.0 (210.0; 247.0)	0.00
	4% FPBS (*n* = 10)	225.0 (206.0; 239,0)	228.0[b] (212.0; 245.0)	223.0 (207.0; 238.0)	223.5 (209.0; 238.0)	221.0 (205.0; 236.0)	0.00
	10% FPBS (*n* = 10)	230.5 (210.0; 237.0)	227.5 (209.0; 234.0)	217.5[c] (201.0; 223.0)	217.5[d] (200.0; 225.0)	216.5[e] (200.0; 222.0)	0.00
	AIc (*n* = 10)	231.0 (221.0; 263.0)	198.0 (182.0; 221.0)	190.0[c] (175.0; 214.0)	188.5[d] (176.0; 212.0)	190.0[e] (176.0; 215.0)	0.00
Left ventricle thickness (mm)	2% FPBS (*n* = 10)	17.2 (13.5; 20.3)	19.1 (14.8; 22.5)	18.6[c] (15.7; 22.3)	20.1[d] (15.4; 22.5)	19.4[e] (15.7; 23.1)	0.00
	4% FPBS (*n* = 10)	18.0 (15.1; 20.3)	19.1 (16.6; 19.4)	20.3[c] (18.5; 21.0)	18.8 (17.5; 20.2)	19.3[e] (18.2; 21.4)	0.01
	10% FPBS (*n* = 10)	18.5 (16.5; 20.1	18.8 (17.0; 20.4)	18.9 (18.1; 20.9)	19.8[d] (18.6; 21.3)	19.0[e] (16.5; 21.5)	0.00
	AIc (*n* = 10)	16.0 (15.1; 17.1)	15.2 (12.7; 18.3)	17.2 (14.7; 19.6)	16.8 (14.8; 18.6)	16.7 (14.3; 18.9)	NS
Right ventricle thickness (mm)	2% FPBS (*n* = 10)	6.1 (4.4; 6.8)	7.2 (7.1; 9.2)	7.8[c] (7.3; 9.1)	8.2[d] (6.8; 9.0)	8.7[e] (8.4; 10.5)	0.00
	4% FPBS (*n* = 10)	5.7 (4.4; 6.5)	6.4 (5.3; 7.1)	7.3[c] (6.0; 7.5)	6.2 (5.4; 8.0)	7.5[e] (5.9; 7.9)	0.00
	10% FPBS (*n* = 10)	6.4 (5.5; 7.3)	6.7 (5.5; 7.4)	7.9[c] (6.5; 8.5)	7.8 (6.7; 8.2)	6.9 (6.3; 7.9)	0.00
	AIc (*n* = 10)	9.4 (7.1; 10.1)	7.5[b] (6.7; 8.6)	8.0[c] (6.5; 9.5)	7.6 (6.8; 9.3)	8.3 (7.8; 9.6)	0.00
Papillary muscle diameter (mm)	2% FPBS (*n* = 10)	5.6 (3.7; 5.8)	6.5 (5.2; 7.2)	6.7[c] (5.0; 7.0)	6.8[d] (5.8; 7.9)	6.8[e] (6.0; 8.0)	0.00
	4% FPBS (*n* = 10)	5.3 (4.7; 6.9)	5.9 (5.1; 7.3)	5.6 (4.5; 7.0)	5.7 (4.6; 7.5)	6.0 (4.8; 8.0)	NS
	10% FPBS (*n* = 10)	6.1 (5.6; 8.6)	6.0 (5.1; 9.5)	6.7 (6.3; 10.1)	7.5[d] (6.6; 10.6)	7.4[e] (6.9; 10.1)	0.00
	AIc (*n* = 10)	4.6 (3.4; 8.3)	5.0 (4.0; 6.2)	4.1 (2.8; 5.5)	5.4 (4.7; 9.0)	4.9 (4.1; 5.9)	NS
Chordae tendineae length (mm)	2% FPBS (*n* = 10)	18.9 (15.8; 20.7)	16.6 (12.2; 22.7)	14.8[c] (10.4; 20.7)	15.6 (12.3; 18.6)	17.8 (13.2; 24.4)	0.01
	4% FPBS (*n* = 10)	18.3 (12.5; 22.4)	11.8[b] (10.5; 14.3)	12.8 (12.0; 17.0)	13.0[d] (8.7; 14.4)	13.7 (12.4; 18.4)	0.00
	10% FPBS (*n* = 10)	12.0 (6.7; 13.8)	8.9 (7.7; 14.1)	11.3 (9.8; 15.2)	12.6 (8.5; 16.8)	14.3 (9.0; 16.7)	NS
	AIc (*n* = 10)	14.7 (13.3; 16.2)	10.6[b] (8.7; 14.7)	10.9[c] (8.8; 12.4)	11.0 (10.4; 12.1)	12.3 (9.8; 17.0)	0.00
Anterior interventricular artery diameter (mm)	2% FPBS (*n* = 10)	2.7 (2.5; 3.0)	2.8 (2.5; 3.4)	2.0 (1.8; 2,2)	2.1[d] (1.6; 2.5)	2.2 (1.6; 2.3)	0.00
	4% FPBS (*n* = 10)	3.2 (2.6; 4.7)	3.1 (2.6; 3.4)	2.8 (2.5; 5.0)	1.9[d] (0.9; 2.4)	2.2[e] (1.3; 3.2)	0.00
	10% FPBS (*n* = 10)	3.3 (3.1; 3.5)	3.7 (2.8; 4.0)	2.7 (2.1; 3.1)	2.6 (2.1; 2.9)	2.3[e] (1.9; 2.4)	0.00
	AIc (*n* = 10)	3.0 (2.7; 3.5)	3.0 (2.2; 3.5)	2.6 (2.3; 2.9)	2.0[d] (1.8; 2.4)	1.6[e] (1.1; 2.0)	0.00
Length on interatrial septum surface (mm)	2% FPBS (*n* = 10)	23.4 (20.0; 24.8)	24.0 (21.5; 26.1)	24.3 (21.7; 25.1)	23.8 (21.7; 26.0)	24.0 (20.8; 25.7)	NS
	4% FPBS (*n* = 10)	24.0 (20.4; 27.1)	22.0 (20.5; 25.6)	23.5 (20.0; 26.5)	23.0[d] (18.8; 25.0)	23.1 (19.1; 25.6)	0.00
	10% FPBS (*n* = 10)	25.6 (24.8; 26.5)	25.4 (24.6; 26.5)	25.3 (23.7; 27.2)	25.5 (23.5; 26.1)	24.8 (23.6; 26.1)	NS
	AIc (*n* = 10)	19.8 (18.3; 21.1)	20.3 (19.4; 22.0)	20.0 (18.5; 23.3)	20.1 (18.5; 20.6)	20.0 (18.6; 20.8)	NS
Angle (°)	2% FPBS (*n* = 10)	90.0 (90.0; 90.0)	92.0 (86.0; 97.0)	91.5 (88.0; 94.0)	93.5 (88.0; 98.0)	91.5 (88.0; 94.0)	NS
	4% FPBS (*n* = 10)	90.0 (90.0; 90.0)	99.0 (93.0; 106.0)	98.0[c] (95.0; 105.0)	101.5[d] (92.0; 106.0)	100.5 (91.0; 103.0)	0.00
	10% FPBS (*n* = 10)	90.0 (90.0; 90.0)	91.5 (84.0; 95.0)	88.5 (86.0; 94.0)	91.5 (89.0; 94.0)	90.5 (86.0; 93.0)	NS
	AIc (*n* = 10)	90.0 (90.0; 90.0)	92.0 (88.0; 97.0)	90.5 (87.0; 94.0)	92.5 (88.0; 97.0)	90.5 (87.0; 95.0)	NS

Notes. AIc: alcoholic formalin solution; FPBS: formaldehyde phosphate-buffered solution; Me: median; *n*: number of samples; Q1 and Q3: lower and upper quartiles.

[a]Friedman test.

[b]Repeated-measures analysis of variance evaluating the 1-week time point score compared with the baseline score, $p < 0.05$.

[c]Repeated-measures analysis of variance evaluating the 3-month time point score compared with the baseline score, $p < 0.05$.

[d]Repeated-measures analysis of variance evaluating the 6-month time point score compared with the baseline score, $p < 0.05$.

[e]Repeated-measures analysis of variance evaluating the 12-month time point score compared with the baseline score, $p < 0.05$.

TABLE 2 Relative (percentage) changes in the measured heart parameters at consecutive time points for samples preserved in different fixatives. The data are presented and compared both with values before the preservation process (A) and between specific time intervals (B)

			One week	3 month	6 month	12 month
Parameter	**Study group**		**Me (Q1; Q3)**	**Me (Q1; Q3)**	**Me (Q1; Q3)**	**Me (Q1; Q3)**
Heart weight	2% FPBS (*n* = 10)	A	3.4% (2.6; 4.3)	0.4% (−0.5; 0.5)	1.3% (0.8; 1.4)	0.0% (−0.4; 0.5)
		B		−3.6% (−3.7; −2.1)	1.0% (0.4; 1.4)	−1.2% (−1.3; −0.9)
	4% FPBS (*n* = 10)	A	2.3% (1.7; 2.7)	0.0% (−0.4; 0.4)	−0.2% (−0.4; 1.1)	−1.3% (−1.8; −0.5)
		B		−2.2% (−2.7; −1.7)	0.0% (−0.4; 0.7)	−1.4% (−1.8; −1.2)
	10% FPBS (*n* = 10)	A	−0.8% (−1.3; −0.4)	−5.3% (−5.9; −4.3)	−4.9% (−6.4; −4.7)	−5.2% (−6.3; −4.7)
		B		−4.5% (−5.0; −3.9)	−0.2% (−0.5; 0.5)	0.0% (−0.5; 0.4)
	Alc (*n* = 10)	A	−16.2% (−17.6; −14.3)	−18.3% (−20.4; −17.5)	−18.7% (−20.0; −16.6)	−18.6% (−20.0; −17.0)
		B		−2.9% (−3.8; −2.3)	−0.1% (−0.6; 0.6)	0.3% (0.0; 1.2)
Left ventricle thickness	2% FPBS (*n* = 10)	A	10.5% (7.9; 13.9)	12.7% (8.5; 17.0)	14.1% (11.3; 15.3)	10.4% (8.4; 16.3)
		B		−1.0% (−2.7; 7.5)	2.8% (−1.9; 5.6)	−1.8% (−3.9; 0.6)
	4% FPBS (*n* = 10)	A	3.6% (−1.5; 8.8)	8.2% (3.4; 22.4)	3.5% (−0.5; 17.1)	6.9% (0.9; 9.9)
		B		3.4% (−0.5; 8.4)	−6.0% (−9.4; 0.0)	6.2% (−0.5; 7.8)
	10% FPBS (*n* = 10)	A	0.9% (−5.5; 10.4)	10.1% (1.4; 14.2)	12.8% (10.4; 16.9)	11.2% (4.3; 13.7)
		B		5.0% (−0.6; 11.8)	1.9% (0.4; 4.0)	−1.7% (−6.6; 1.2)
	Alc (*n* = 10)	A	−2.6% (−20.2; −1.2)	3.2% (−5.1; 17.5)	2.6% (−2.5; 15.4)	2.0% (−5.1; 15.8)
		B		6.9% (3.0; 14.4)	−1.8% (−5.1; 2.7)	0.8% (−1.7; 2.2)
Right ventricle thickness	2% FPBS (*n* = 10)	A	21.7% (16.4; 35.3)	33.0% (19.7; 47.5)	36.6% (23.0; 53.5)	39.1% (33.7; 53.5)
		B		5.7% (1.4; 9.6)	0.7% (−6.7; 4.8)	7.2% (0.0; 12.3)
	4% FPBS (*n* = 10)	A	17.4% (7.0; 24.4)	29.2% (17.6; 37.9)	20.3% (7.4; 31.7)	32.5% (21.5; 42.5)
		B		13.4% (3.9; 17.6)	−10.3% (20.0; −4.7)	17.1% (−1.3; 26.8)
	10% FPBS (*n* = 10)	A	−0.8% (−5.1; 19.0)	18.6% (14.6; 24.8)	13.8% (11.9; 40.8)	9.8% (−1.7; 20.9)
		B		14.7% (4.8; 18.7)	−3.4% (−4.5; −0.2)	−3.6% (−12.2; 0.5)
	Alc (*n* = 10)	A	−16.1% (−20.0; −13.1)	−14.5% (−20.0; −5.3)	−13.8% (−23.6; −3.0)	−3.2% (−7.4; 2.7)
		B		5.4% (−7.0; 9.7)	8.0% (−8.8; 11.4)	11.2% (0.0; 22.1)
Papillary muscle diameter	2% FPBS (*n* = 10)	A	23.7% (17.9; 44.8)	32.3% (16.7; 41.4)	33.5% (24.0; 73.9)	35.8% (32.1; 65.2)
		B		2.4% (−2.8; 7.8)	3.0% (−2.8; 12.9)	2.6% (−1.5; 6.5)
	4% FPBS (*n* = 10)	A	18.7% (−1.7; 38.2)	6.6% (−13.6; 30.4)	18.9% (−6.7; 41.7)	22.7% (−1.2; 45.5)
		B		−4.8% (−7.9; −3.4)	−0.9% (−2.9; 14.3)	7.2% (4.3; 8.8)
	10% FPBS (*n* = 10)	A	2.9% (−7.6; 8.7)	17.4% (0.9; 19.0)	15.6% (3.8; 27.6)	17.3% (8.5.; 31.6)
		B		9.7% (6.9; 18.8)	4.1% (−0.1; 6.9)	1.5% (−1.3; 4.5)
	Alc (*n* = 10)	A	−8.6% (−21.6; 21.2)	−14.1% (−30.4; 19.6)	3.3% (−22.7; 17.6)	8.9% (−20.5; 15.7)
		B		−3.4% (−23.9; 6.4)	29.0% (0.0; 77.4)	−2.5% (−14.8; 6.3)
Chordae tendineae length	2% FPBS (*n* = 10)	A	−12.2% (−16.0; −3.5)	−11.9% (−27.4; −5.6)	−12.6% (−21.4; −8.9)	−8.2% (−13.0; 17.9)
		B		−4.4% (−13.5; 4.9)	4.6% (−5.0; 11.6)	8.7% (5.2; 25.4)
	4% FPBS (*n* = 10)	A	−30.0% (−42.1; −20.2)	−22.2% (−27.4; −1.8)	−39.2% (−47.9; −26.6)	−13.7% (−19.5; 0.4)
		B		9.2% (4.4; 18.9)	−21.1% (−33.1; −14.5)	33.6% (22.8; 64.0)
	10% FPBS (*n* = 10)	A	−6.0% (−24.4; 4.9)	2.3% (−6.6; 23.0)	12.6% (−10.8; 26.9)	22.6% (0.1; 31.1)
		B		14.5% (−10.9; 31.0)	1.7% (−10.9; 15.0)	1.0% (−1.8; 7.4)
	Alc (*n* = 10)	A	−25.0% (−41.9; −12.4)	−28.5% (−34.0; −14.5)	−19.6% (−34.6; −9.0)	−12.0% (−30.7; −4.2)
		B		−2.9% (−9.1; 1.6)	4.9% (−2.8; 18.2)	1.8% (−6.0%; 17.3)

(*Continues*)

TABLE 2 (continued)

Parameter	Study group		One week Me (Q1; Q3)	3 month Me (Q1; Q3)	6 month Me (Q1; Q3)	12 month Me (Q1; Q3)
Anterior interventricular artery diameter	2% FPBS (*n* = 10)	A	4.8% (−11.1; 17.2)	−24.5% (−44.4; −18.2)	−21.9% (−44.4; −15.4)	−15.8% (−46.7; −4.8)
		B		−27.5% (−37.5; −23.5)	0.0% (−15.8; 21.4)	2.3% (−8.0; 16.0)
	4% FPBS (*n* = 10)	A	−4.9% (−12.9; 21.9)	21.7% (11.7; 30.0)	−34.3% (−55.3%; −24.4)	−32.4% (−54.3; −12.2)
		B		25.3% (−3.1; 33.4)	−45.7% (−66.2; −27.4)	−3.6% (−7.7; 51.2)
	10% FPBS (*n* = 10)	A	−5.9% (−20.9; 17.5)	−21.0% (−33.3; −4.6)	−27.4% (−35.6; −10.8)	−34.5% (−40.0; −26.2)
		B		−19.4% (−27.2; −12.5)	−2.9% (−12.9; 0.2)	−8.8% (−17.2; −0.1)
	Alc (*n* = 10)	A	−8.0% (−18.5; 3.6)	−14.5% (−28.6; −4.2)	−30.8% (−37.5; −26.3)	−45.9% (−60.7; −29.2)
		B		−13.5% (−23.3; 6.0)	−15.5% (−34.8; 5.0)	−22.0% (−42.1; −7.7)
Length on interatrial septum surface	2% FPBS (*n* = 10)	A	−2.0% (−3.3; 5.6)	0.9% (−2.7; 8.1)	−0.9% (−3.0; 5.6)	−0.6% (−4.1; 5.6)
		B		1.6% (−1.9; 2.5)	−1.4% (−2.5; 0.9)	−0.2% (−1.2; 0.5)
	4% FPBS (*n* = 10)	A	−0.3% (−10.8; 1.8)	−2.1% (−6.4; 2.0)	−5.4% (−8.1; −0.5)	−4.4% (−9.4; 0.9)
		B		−1.7% (−2.4; −0.4)	−2.9% (−5.7; −2.4)	1.1% (−0.7; 2.8)
	10% FPBS (*n* = 10)	A	−0.5% (−3.9; 2.2)	0.0% (−3.4; 4.4)	−0.2% (−6.2; 2.6)	0.1% (−6.6; 1.8)
		B		0.4% (−1.2; 1.0)	−0.2% (−2.3; 0.3)	−0.2% (−0.8; 1.6)
	Alc (*n* = 10)	A	2.5% (−4.4; 11.7)	0.9% (−2.9; 9.3)	−1.0% (−3.9; 2.7)	−0.1% (−2.1; 6.0)
		B		−0.7% (−4.9; 2.2)	0.8% (−1.3; 4.7)	0.9% (−1.7; 1.6)
Angle	2% FPBS (*n* = 10)	A	2.2% (−4.4; 7.8)	1.7% (−2.2; 4.4)	3.9% (−2.2; 8.9)	1.7% (−2.2; 4.4)
		B		−0.5% (−3.8; 2.3)	2.7% (−1.1; 3.5)	−1.2% (−4.1; 0.0)
	4% FPBS (*n* = 10)	A	10.0% (3.3; 17.8)	8.9% (5.6; 16.7)	12.8% (2.2; 17.8)	11.7% (1.1; 14.4)
		B		1.1% (−0.9; 4.0)	−0.5% (−3.2; 3.1)	−1.0% (−3.4; 0.0)
	10% FPBS (*n* = 10)	A	1.7% (−6.7; 5.6)	−1.7% (−4.4; 4.4)	1.7% (−1.1; 4.4)	0.6% (−4.4; 3.3)
		B		−1.1% (−3.2; 3.5)	3.4% (−2.1; 5.8)	−0.5% (−2.2; 0.0)
	Alc (*n* = 10)	A	2.2% (−2.2; 7.8)	0.6% (−3.3; 4.4)	2.8% (−2.2; 7.8)	0.6% (−3.3; 5.6)
		B		−1.6% (−3.9; −1.0)	−3.1% (−1.1; 4.6)	−1.6% (−4.1; −1.0)

Notes. A—relative (percentage) changes of parameters in particular time intervals, always compared with results before fixation (baseline); baseline–1 week, baseline–3 month, baseline–6 month, baseline–12 month.
B—relative (percentage) changes of parameters in time intervals in the point-to-point comparison; 1 week–3 month, 3–6 month and 6–12 month.
Alc: alcoholic formalin solution; FPBS: formaldehyde phosphate-buffered solution; Me: median; *n*: number of samples; Q1 and Q3: lower and upper quartiles.

$p < 0.05$) were observed. In 4% FPBS, significant changes in three parameters were noticed: an increase in the left (+8.2%, $p < 0.05$) and right ventricle thickness (+29.2%, $p < 0.05$), and angle (+8.9%, $p < 0.05$). In a 10% FPBS, only the heart weight (−5.3%, $p < 0.05$) and right ventricle thickness (+18.6%, $p < 0.05$) had changed significantly. The fixation in alcoholic formalin solution led to reduction of heart weight (−18.3%, $p < 0.05$), right ventricle thickness (−14.5%, $p < 0.05$) and chordae tendineae (−28.5%, $p < 0.05$). The length on interatrial septum and left anterior descending artery diameter were not significantly changed in all tested solutions. The 10% FPBS caused significant changes in the smallest number of parameters (2 parameters: heart weight and right ventricle thickness), while the 2% FPBS affected the greatest number of dimensions (4 parameters: left and right ventricle thickness, papillary muscle diameter, chordae tendineae length).

3.2 | Baseline—6-month changes

After six months, the most noticeable significant changes compared to the baseline score was observed in 2% FPBS: increase in muscle tissue thickness (left ventricle thickness: +14.1%, $p < 0.05$; right ventricle: +36.6%, $p < 0.05$; papillary muscle diameter: +33.5%, $p < 0.05$), and decrease in artery diameter (−21.9%, $p < 0.05$). Also, the 4% FPBS was a solution that greatly affected heart dimensions and, in particular, led to an increase in angle (+12.8%, $p < 0.05$), decrease in chordae tendineae length (−39.2%, $p < 0.05$], decrease in artery diameter (−34.3%, $P < 0.05$) and slight decrease in length on interatrial septum surface (−5.4%, $p < 0.05$). The fixation in a 10% FPBS significantly reduced the heart weight (−4.9%, $p < 0.05$] and increased muscle thickness (left ventricle thickness: +12.8%, $p < 0.05$; papillary muscle diameter: +15.6%, $p < 0.05$). A decrease in heart

weight (−18.7%, $p < 0.05$) and artery diameter (−30.8%, $p < 0.05$) was observed in alcoholic formalin solution and this fixative was also the one that affected the least parameters.

3.3 | Baseline—12-month changes

After 12 months, an increase in ventricle thickness in 2% FPBS (left ventricle thickness: +10.4%, $p < 0.05$; right ventricle thickness: +39.1%, $p < 0.05$) 4% FPBS (left ventricle thickness: +6.9%, $p < 0.05$; right ventricle thickness: +32.5%, $p < 0.05$) and 10% FPBS (left ventricle thickness: +11.2%, $p < 0.05$) was noted. The heart weight decreased in a 10% FPBS (−5.2%, $p < 0.05$) and alcoholic formalin solution (−18.6%, $p < 0.05$). After a 1-year observation, papillary muscle diameter decreased in comparison with the baseline score in 2% FPBS (+35.8%, $p < 0.05$) and 10% FPBS (+17.3%, $p < 0.05$). The long-term fixation also noticeably influenced the artery diameter, leading to a decrease in 4% FPBS (−32.4%, $p < 0.05$), 10% FPBS (−34.5%, $p < 0.05$) and alcoholic formalin solution (−45.9%, $p < 0.05$]. Changes in chordae tendineae length, angle and marked length on interatrial septum surface were not significant in any of the tested solutions. The alcoholic formalin solution caused significant changes in the smallest number of parameters (2 parameters: heart weight, anterior interventricular artery diameter), while the 10% FPBS affected the greatest number of dimensions (4 parameters: heart weight, left ventricle thickness, papillary muscle diameter, anterior interventricular artery diameter).

3.4 | Short-time–long-time fixation changes

The heart weight decreased in all tested solutions between the 1-week and 3-month points. Longer fixing does not cause any dynamic changes (Figure 1a).

The left ventricle thickness increases in a 4% FPBS and alcoholic formalin and stabilize after 6 months of fixation. In 2% FPBS left ventricle thickness fluctuating during long-time observation; moreover, the 10% FPBS caused linear increase in thickness during the first 6 months and a reduction was noticed after 1-year fixation (Figure 1b).

The right ventricle thickness slightly and continuously increases during fixation in alcoholic formalin solution. In aqueous formaldehyde solutions 1-year fixation caused a significant increase in right ventricle thickness in 2% and 4% FPBSs (Figure 1c).

Papillary muscle diameter continuously increased in 2% and 10% FPBSs. In 4% FPBS, we noticed an initial decrease in the thickness, with slow increase to 1-week point value. For alcoholic formalin solution, we observed a noticeable increase after 6 months but in the next 6 months, the changes slightly reversed (Figure 1d).

The length of chordae tendineae showed an increasing trend in the 2%, 10% FPBS and alcoholic formalin solution. In 4% FPBS, results fluctuated (Figure 1e).

The artery diameter decreased in a long-term fixation in every tested solution, except 4% FPBS, where after a large increase at the 3-month point, we noticed significant shrinkage of the vessels (Figure 1f).

The length on the interatrial surface did not change in 2% and 10% FPBS during the observation period. In 4% FPBS, the results slightly fluctuated with a decreasing tendency, also the alcoholic formalin solution caused a decrease in the length (Figure 1g).

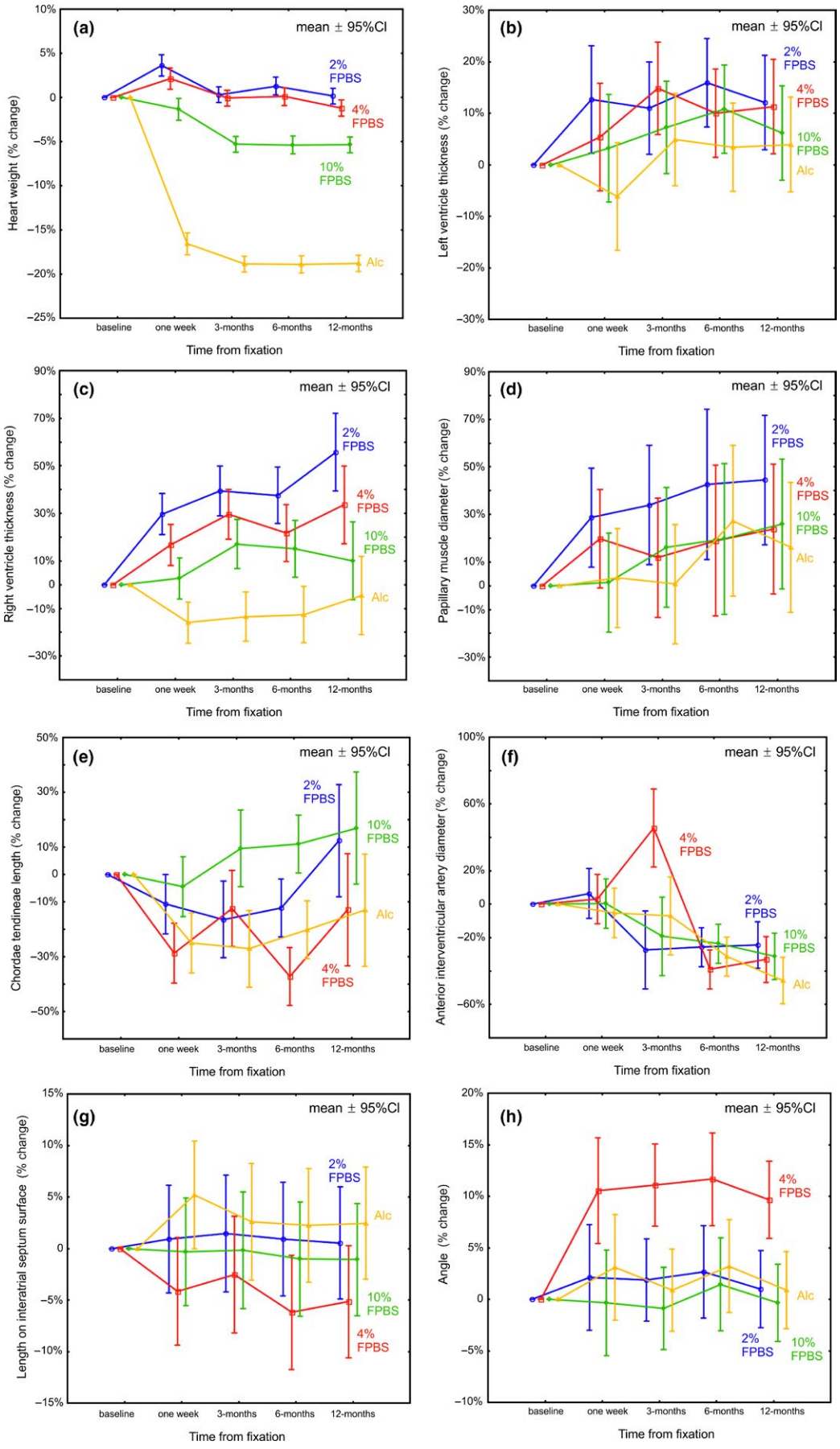
The difference in the angle size was not significant during long-term observation (Figure 1h).

4 | DISCUSSION

The fact that computed tomography, echocardiography and other imaging techniques are rapidly becoming the golden standard for measurement of cardiac dimensions in a clinical setting is undeniable (Balli, Aytemir, & Karcaaltincaba, 2012). However, macroscopic anatomy is still the base for imaging and clinical studies. This is evidenced by the countless number of morphometric studies from recent years (Ciuk, Janas, & Klimek-Piotrowska, 2018; Kucybała, Ciuk, & Klimek-Piotrowska, 2018). The formaldehyde-based fixation is an essential step for all morphometric studies and allowing multiple assessment moments for up to a few years (Hołda et al., 2017). Moreover, human heart libraries, repositories with hundreds of formaldehyde-fixed human heart specimens, exist worldwide (Iaizzo, 2016). Despite this, there is a lack of detailed information about how long-term fixation and storage of cardiac tissue affect tissue dimensions and shape. Moreover, the type of changes, caused by the long-term fixation process, was never studied in detail.

Our study reveals that not only the first step of fixation, greatly associated with the penetration of the solution into the tissue, may significantly influence the results of the measurements (Hołda et al., 2016, 2017). The continuous interaction between the tissue and the fixative may also play a role in the further fixation process. Initial changes may be either exacerbated or reversed. In our previous study, we investigated the influence of different fixation protocols on the preservation and dimensions of cardiac tissue during a short-term (1-week) observation. Two conclusions were made in an earlier study: (a) 10% FPBS is the best fixative among all studied because it caused little alterations in tissue dimensions; (b) measurements should be taken at least 1 week after preservation as many parameters exhibited the smallest changes when compared with non-preserved samples (Hołda et al., 2016). Results of our two studies lead us to the general conclusion that cardiac tissue demonstrates a fast and multidirectional change within the first days of fixation. Afterwards, the tissue is additional fixated, showing a much slower change in tissue dimensions. Also, stabilization of certain parameters

FIGURE 1 Graphs of means with 95% confidence intervals (CIs) of the percentage changes of the measured morphometric parameters of the heart samples preserved in different fixatives at consecutive time points. 2% formaldehyde phosphate-buffered solution (FPBS; $n = 10$); 4% FPBS ($n = 10$); 10% FPBS ($n = 10$); alcoholic formalin solution (Alc; $n = 10$)


(a)
mean ± 95%CI
Heart weight (% change)
2% FPBS
4% FPBS
10% FPBS
Alc
baseline
one week
3-months
6-months
12-months
Time from fixation
(b)
mean ± 95%CI
Left ventricle thickness (% change)
(c)
mean ± 95%CI
Right ventricle thickness (% change)
(d)
mean ± 95%CI
Papillary muscle diameter (% change)
(e)
mean ± 95%CI
Chordae tendineae length (% change)
(f)
mean ± 95%CI
Anterior interventricular artery diameter (% change)
(g)
mean ± 95%CI
Length on interatrial septum surface (% change)
(h)
mean ± 95%CI
Angle (% change)

may be detected in long-term observation, especially for heart weight and atrial structures.

As expected, both in short- and long-term observation, thickening of the cardiac muscle in formaldehyde-based fixatives was observed (Cutts, 1988; Hołda et al., 2016). It is interesting that the biggest changes were not associated with the thickest parts of the heart muscle (i.e., the left ventricle wall) but parts with moderate thickness (i.e., the right ventricle wall and papillary muscles). Once again, the greatest changes were observed in 2% and 4% FPBSs. The 10% FPBS and alcoholic formalin solution caused significantly smaller changes in muscular tissue thickness; however, because the latter makes the tissue more fragile and breakable, the 10% FPBS should be preferred for studies investigating ventricular anatomy.

The short-term observation reveals that the diameter of the artery was not subjected to a significant change. However, in all investigated solutions, longer fixation caused a slow but continuous and significant decrease in the diameter (Hołda et al., 2016). The 4% FPBS is the only solution that initially caused a relaxation of the vessel but, in the long run, the effect (shrinkage of the vessel) was similar for all fixatives. The smallest changes were associated with the lowest concentration of the FPBS. Therefore, the 2% FPBS should be preferred for studies assessing arterial blood vessels of the heart.

When looking at the atrial tissue, the observed changes are so small (below 5%, close to human bias) that they could be ignored. However, 4% FPBS (statistically significant decreasing atrial dimensions) and alcoholic formalin solutions (increase dimensions and hardened the tissue) should not be used when assessing atrial structures.

The main limitation of our study was the use of animal tissue instead of human tissue. However, a swine heart has many anatomical similarities with a human heart and is widely used as a model in cardiovascular research (Crick, Sheppard, Ho, Gebstein, & Anderson, 1998; Hill & Iaizzo, 2015; Stanton & Mersmann, 1986; Ye et al., 1994). The animal tissue used in this study did not differ in any of the studied morphological aspects that were studied compared to human tissue. It is expected that the changes, induced by the fixation process of porcine and human hearts, will demonstrate several similarities.

In conclusion, 1-year fixation significantly changed cardiac tissue dimension and shape compared to the nonpreserved samples in all tested solutions. When comparing the short-term to 1-year fixation, observed changes were smaller, but they should not be overlooked; some corrections may be necessary. Different fixatives should be used depending on the character of the planned measurements. The 10% FPBS should be preferred for studies investigating ventricular anatomy, while the 2% FPBS should be preferred for studies assessing coronary arteries. The 2% and 10% FPBS should be favoured for atrial tissue measurements.

CONFLICT OF INTEREST

Nothing to declare.

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