
8 ADDENDA

8.1 Review of surgical procedure

8.2 Tables used for statistical analysis

8.1 Review of surgical procedure

All the biochemical and histological preparatory laboratory procedures and the clinical experimental procedures below have been previously accomplished and published by Ripamonti et al. (1987, 1989).

Selection of animals

Two clinically healthy adult male baboons (*Papio ursinus*) with normal haematological profile were selected from the primate colony of the University of the Witwatersrand, Johannesburg. Skeletal maturity was assessed by radiographic evidence of closure of the distal epiphysis of the radii and ulnae. Following standard quarantine procedures, the baboons were housed individually in suspended wire-mesh squeeze-back cages in the nonhuman primate unit of the Central Animal Service of the University approximately 1750 m above sea level. The cages were in rooms kept under a slight negative pressure (-25 KPa) with controlled ventilation (18 filtered air changes/hour), temperature of 22° C $\pm$ 2° C and humidity of approximately 40 % $\pm$ 10 %.

Preparation of the allogeneic fibrin-fibronectin protein concentrate

Two adult male chacma baboons (*Papio ursinus*) were immobilized with intramuscular phencyclidine hydrochloride at a dosage of 1 mg/kg body weight. General anaesthesia was obtained by intravenous administration of sodium pentobarbitone at a dosage of 6 mg/kg body weight. The femoral arteries were surgically exposed, cannulated, and approximately 900 ml of blood was obtained from each animal. The blood was then

placed into 250 ml sterile vials containing 20 000 KIU (Kallidinogenase Inactivator Units) of bovine aprotinin and 4 % sodium citrate, and then centrifuged at 2000 rpm for 20 minutes. The plasma was stored at -20° C in sterile vials until fibrinogen, fibronectin, factor XIII and plasminogen were cryoprecipitated to form a lyophilized allogeneic protein concentrate. The final preparation of the AFFP was done in the Immuno AG Laboratories, Vienna.

Surgical model and experimental design

Both selected baboons had intact dentition and generalized gingivitis without loss of periodontal attachment. The animals were anaesthetized and the airway was maintained patent by endotracheal intubation. Buccal intrasulcular incisions were done around the mandibular first and second molars to elevate a flap using aseptic techniques. Then, the molars were hemisected in a bucco-lingual direction at the site of the furcation (Fig. 2).

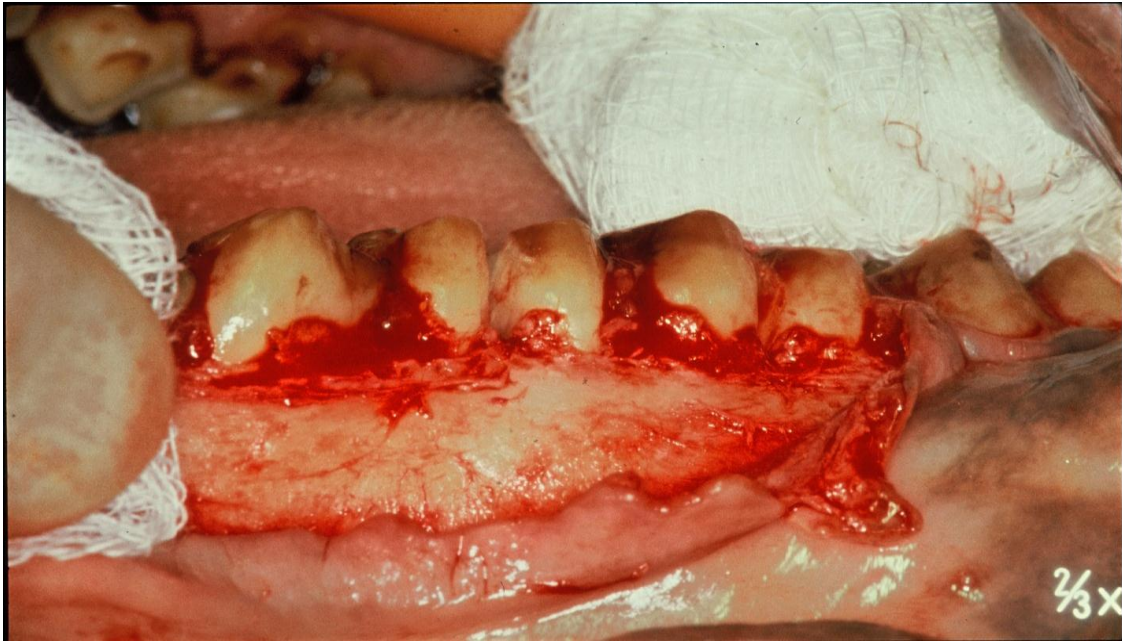


Figure 11 A flap is reflected to prepare recipient alveoli in the buccal alveolar and basal bone of the right side of the mandible in animal 1. First and second molars are hemisected.

A buccal vertical releasing incision anterior to the first premolars allowed further flap reflection to prepare the recipient alveoli (Fig.11). Before the transplantation of the experimental molar roots four alveoli, with a size slightly superior to the size of the roots to be transplanted, were prepared in the alveolar and basal bone adjacent to the extraction sites (Fig.12). The alveoli were prepared as beds parallel to the cortical surface to allow half of the thickness of the roots to be in contact with the basal bone while the other half was in contact with the overlying lining alveolar mucosa.

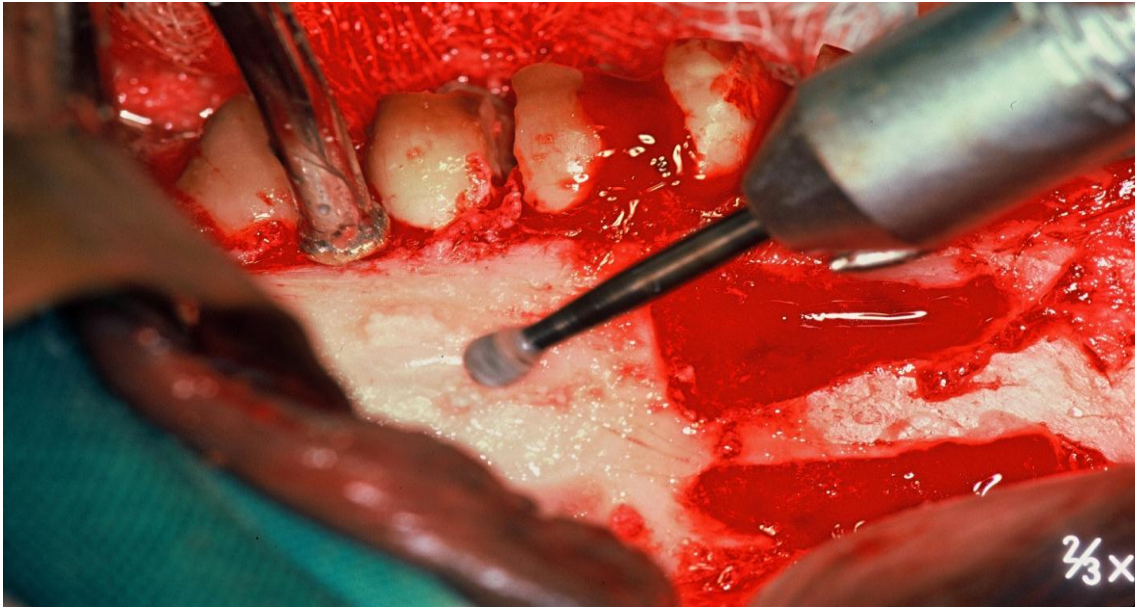


Figure 12 Preparation of the recipient alveoli in the right side of the mandible of animal 1. The alveoli are designated as A1PUR, A1PLR, A1AUR, A1ALR (see abbreviations on page x)

Then, the hemisected molars were extracted using forceps with minimum trauma, thus reducing the morbidity. Following extraction, each root underwent pulpectomy and thorough irrigation of the canal with a sterile saline solution. The coronal two thirds of the root were planed to remove the remnants of the periodontal ligament and the cementum. Measurements were then taken from the cemento-enamel junction using a periodontal probe to ensure that equal amounts of cementum and periodontal ligament were removed from the experimental and control roots. A notch was cut with a small round bur at the junction between the planed and non-planed surfaces (Fig. 13).



Figure 13 A notch separates the planed from the non-planed root surface

Demineralisation of the planed surfaces was done with a saturated solution of citric acid for 3 minutes. The hemisected crowns were separated from the experimental roots and coated with the AFFP activated by bovine thrombin (Immuno AG, Vienna) which allows the fibrinogen to be converted into fibrin as in the physiological coagulation process (Figs 14-17). The volume of activated AFFP used for each animal was 0.5 ml for the biochemical coating. The AFFP allowed for a rapid biochemical adhesion between the roots and the surrounding soft and hard tissues. The control roots were transplanted without using AFFP but the root surfaces were also demineralised.

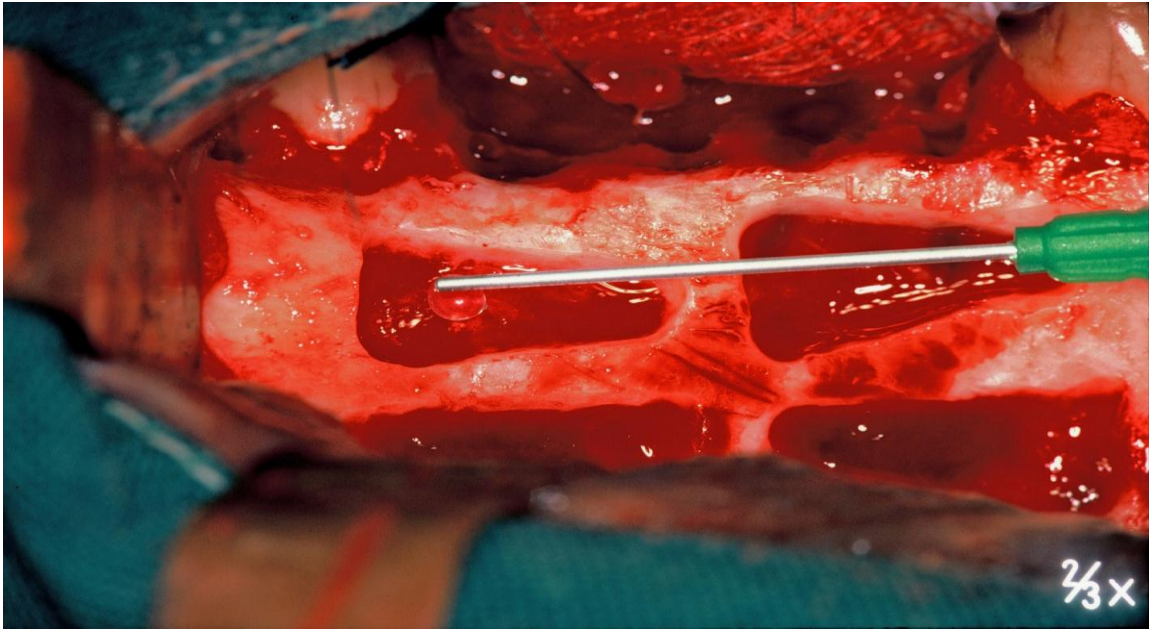


Figure 14 Injection of AFFP in the recipient alveoli

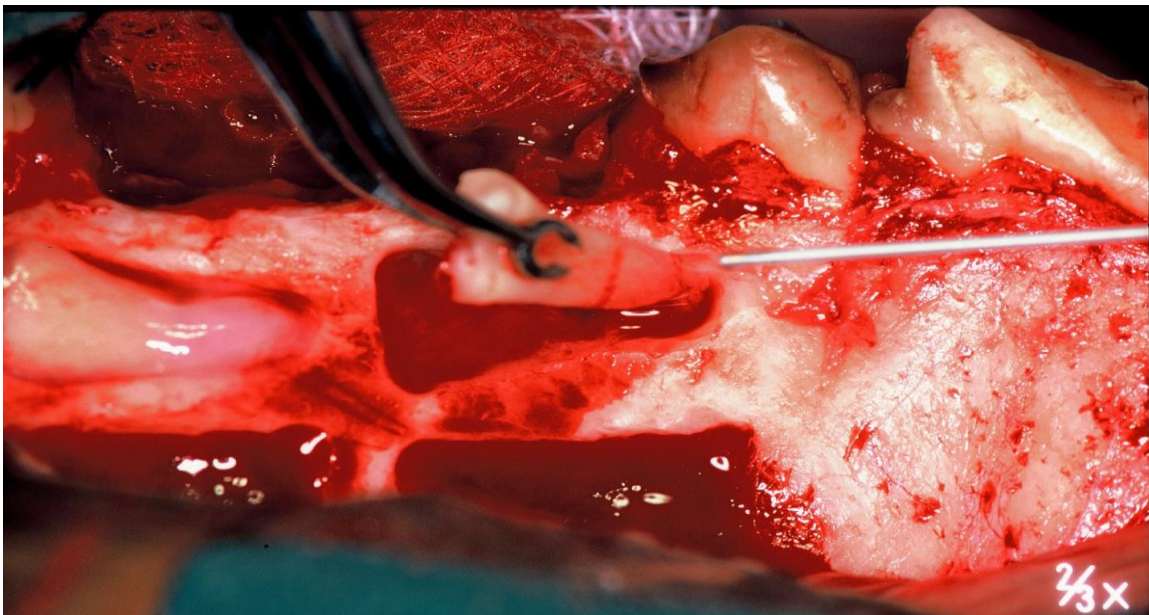


Figure 15 Placement of the resected roots in the recipient alveoli. The experimental roots are further coated with AFFP.



Figure 16 Resected roots embedded in the alveoli

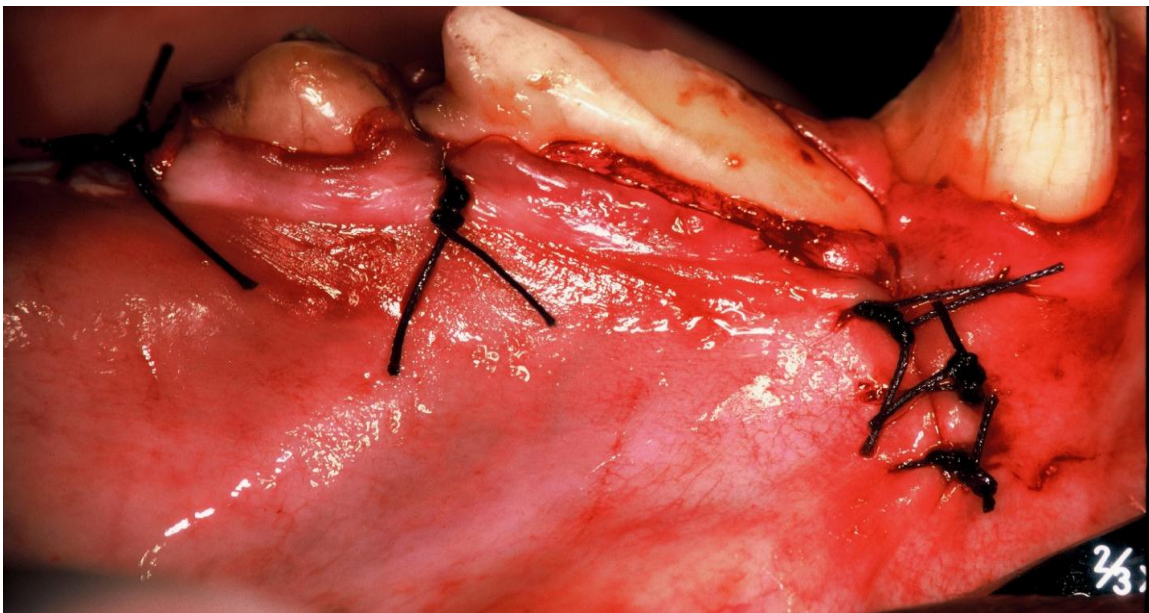


Figure 17 Closure of the operated site

The animals were observed daily after the operation. For the first 10 days of healing, the animals were fed a soft diet consisting of basic proteins, carbohydrates, fats, fibres, calcium, iron, phosphates and vitamins. The sutures were removed from the operated sites after 10 days and a strict plaque control regime was implemented consisting of tooth brushing and topical application of 0.2 % solution of chlorhexidine gluconate twice a week for the duration of the experiment.

Tissue harvest and histology

Fifty-five days after surgery, the animals were euthanized with an intravenous overdose of sodium pentobarbitone. The tissues at the operated sites were then fixed by retrograde aortic perfusion with an isotonic saline solution containing heparin (1000 U/kg body weight) followed by 10 % neutral buffered formaldehyde. The mandibular molar regions were harvested and radiographed. Each specimen was then cut and consisted of one molar root, cortical and medullary basal bone and lining alveolar mucosa. The blocks were then radiographed, decalcified in formic acid-sodium citrate solution and double embedded in colloidin and paraffin wax. The experimental teeth (citric acid demineralization plus AFFP) and the control teeth (citric acid demineralization only) have now been assessed for histology and histomorphometry.

8.2 Tables used for statistical analysis

Table 1 (region 1 NA)

1 NA									
Effect	Level of factor	Level of factor	Level of factor	n°	1 NA propn Mean in %	1 NA propn Std.Dev . in %	1 NA propn Std.Err in %	1 NA propn -95 %	1 NA propn 95 %
Total				68	5.7	15.9	1.9	1.8	9.5
Animal	1			36	7.8	19.0	3.2	1.4	14.2
Animal	2			32	3.3	11.3	2.0	-0.8	7.4
Location	P			42	5.9	16.7	2.6	0.8	11.1
Location	A			26	5.2	14.9	2.9	-0.8	11.2
Treatment	Ex			35	5.0	14.2	2.4	0.1	9.9
Treatment	C			33	6.4	17.7	3.1	0.1	12.7
Animal*Location	1	P		24	6.0	18.3	3.7	-1.7	13.7
Animal*Location	1	A		12	11.3	20.6	6.0	-1.8	24.5
Animal*Location	2	P		18	5.9	14.7	3.5	-1.5	13.2
Animal*Location	2	A		14	0.0	0.0	0.0	0.0	0.0
Animal*Treatment	1	Ex		23	7.6	17.1	3.6	0.2	15.0
Animal*Treatment	1	C		13	8.1	22.7	6.3	-5.6	21.8
Animal*Treatment	2	Ex		12	0.0	0.0	0.0	0.0	0.0
Animal*Treatment	2	C		20	5.3	14.1	3.1	-1.3	11.9
Location*Treatment	P	Ex		19	2.1	8.9	2.1	-2.3	6.4
Location*Treatment	P	C		23	9.2	20.7	4.3	0.2	18.1
Location*Treatment	A	Ex		16	8.5	18.4	4.6	-1.3	18.3
Location*Treatment	A	C		10	0.0	0.0	0.0	0.0	0.0
Animal*Location*Treatment	1	P	Ex	13	3.0	10.8	3.0	-3.5	9.5
Animal*Location*Treatment	1	P	C	11	9.6	24.5	7.4	-6.9	26.0
Animal*Location*Treatment	1	A	Ex	10	13.6	22.1	7.0	-2.2	29.4
Animal*Location*Treatment	1	A	C	2	0.0	0.0	0.0	0.0	0.0
Animal*Location*Treatment	2	P	Ex	6	0.0	0.0	0.0	0.0	0.0
Animal*Location*Treatment	2	P	C	12	8.8	17.5	5.1	-2.3	19.9
Animal*Location*Treatment	2	A	Ex	6	0.0	0.0	0.0	0.0	0.0
Animal*Location*Treatment	2	A	C	8	0.0	0.0	0.0	0.0	0.0

Table 2 Untransformed data (1 NA)

Effect	Univariate results for each DV (SS data for analysis) Sigma-restricted parameterization Effective hypothesis decomposition				
	Degr. of freedom	1 NA propn SS	1 NA propn MS	1 NA propn F	1 NA propn p
Intercept	1	0.09344	0.09344	3.692918	0.059397
Animal	1	0.02306	0.02306	0.911387	0.343577
Location	1	0.004602	0.004602	0.181874	0.671293
Treatment	1	0.000238	0.000238	0.009415	0.923026
Animal*Location	1	0.007394	0.007394	0.292209	0.59081
Animal*Treatment	1	0.019151	0.019151	0.756902	0.387767
Location*Treatment	1	0.064149	0.064149	2.535288	0.116582
Animal*Location*Treatment	1	0.009891	0.009891	0.39091	0.534192
Error	60	1.518145	0.025302		
Total	67	1.689684			

Table 3 Ranked data (1 NA)

Effect	Univariate results for each DV (SS data for analysis) Sigma-restricted parameterization Effective hypothesis decomposition				
	Degr. of freedom	1 NA rank SS	1 NA rank MS	1 NA rank F	1 NA rank p
Intercept	1	62679.43	62679.43	408.3534	0
Animal	1	101.1	101.1	0.6587	0.420233
Location	1	108.26	108.26	0.7053	0.40435
Treatment	1	34.03	34.03	0.2217	0.639451
Animal*Location	1	77.8	77.8	0.5069	0.479251
Animal*Treatment	1	179.34	179.34	1.1684	0.284064
Location*Treatment	1	610.54	610.54	3.9777	0.050657
Animal*Location*Treatment	1	30.07	30.07	0.1959	0.659616
Error	60	9209.59	153.49		
Total	67	10769			

Figure 1 1 NA

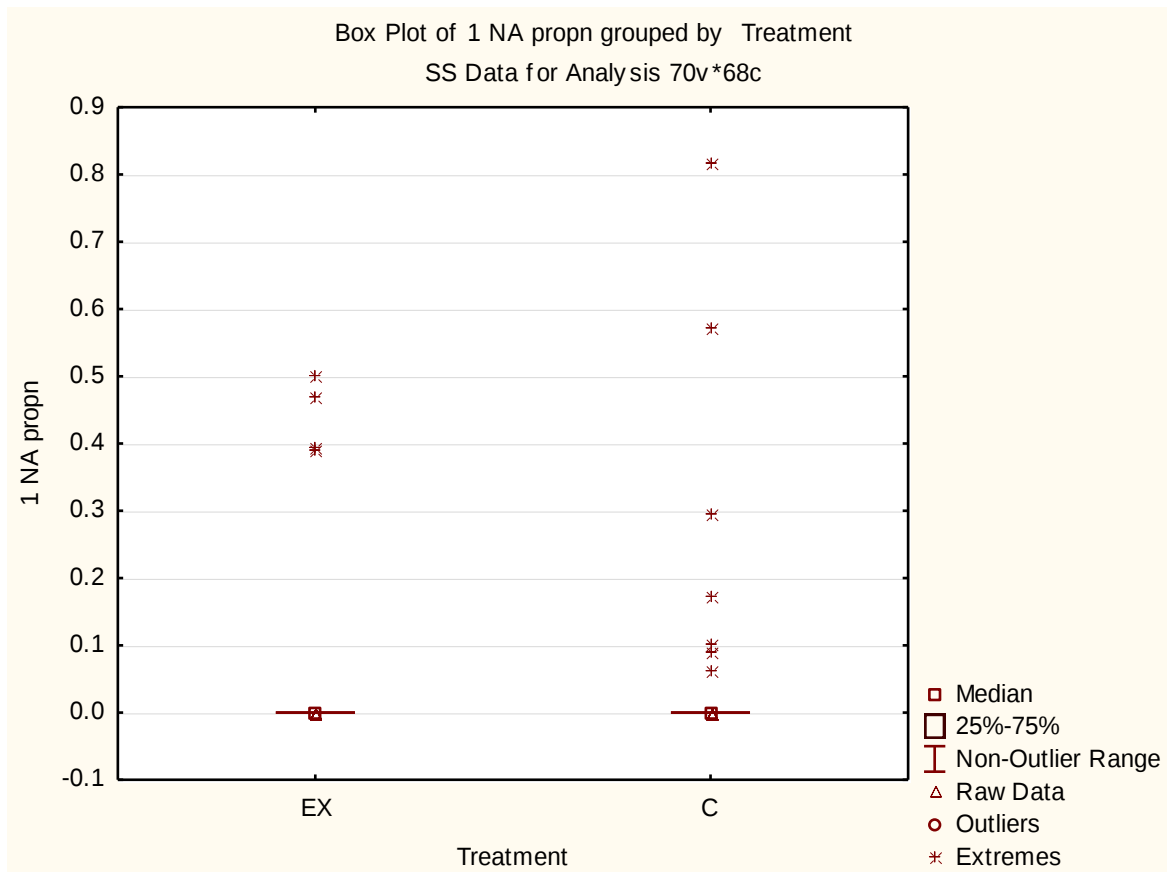


Table 4 (region 2 NA)

2 NA									
Effect	Level of factor	Level of factor	Level of factor	n°	1 NA propn Mean in %	1 NA propn Std.Dev. in %	1 NA propn Std.Err in %	1 NA propn -95 %	1 NA propn 95 %
Total				68	5.6	16.4	2.0	1.7	9.6
Animal	1			36	10.1	21.5	3.6	2.8	17.4
Animal	2			32	0.6	3.3	0.6	-0.6	1.8
Location	P			42	6.7	16.9	2.6	1.5	12.0
Location	A			26	3.8	15.8	3.1	-2.6	10.2
Treatment	Ex			35	2.8	13.7	2.3	-1.9	7.5
Treatment	C			33	8.6	18.7	3.3	2.0	15.2
Animal*Location	1	P		24	11.0	21.2	4.3	2.1	20.0
Animal*Location	1	A		12	8.3	23.0	6.6	-6.3	22.9
Animal*Location	2	P		18	1.0	4.5	1.0	-1.2	3.3
Animal*Location	2	A		14	0.0	0.0	0.0	0.0	0.0
Animal*Treatment	1	Ex		23	4.3	16.8	3.5	-2.9	11.6
Animal*Treatment	1	C		13	20.3	25.6	7.1	4.9	35.8
Animal*Treatment	2	Ex		12	0.0	0.0	0.0	0.0	0.0
Animal*Treatment	2	C		20	0.9	4.2	0.9	-1.0	2.9
Location*Treatment	P	Ex		19	0.0	0.0	0.0	0.0	0.0
Location*Treatment	P	C		23	12.3	21.4	4.5	3.1	21.6
Location*Treatment	A	Ex		16	6.2	20.1	5.0	-4.5	16.9
Location*Treatment	A	C		10	0.0	0.0	0.0	0.0	0.0
Animal*Location*Treatment	1	P	Ex	13	0.0	0.0	0.0	0.0	0.0
Animal*Location*Treatment	1	P	C	11	24.0	26.2	7.9	6.4	41.7
Animal*Location*Treatment	1	A	Ex	10	10.0	25.1	7.9	-8.0	27.9
Animal*Location*Treatment	1	A	C	2	0.0	0.0	0.0	0.0	0.0
Animal*Location*Treatment	2	P	Ex	6	0.0	0.0	0.0	0.0	0.0
Animal*Location*Treatment	2	P	C	12	1.6	5.5	1.6	-1.9	5.0
Animal*Location*Treatment	2	A	Ex	6	0.0	0.0	0.0	0.0	0.0
Animal*Location*Treatment	2	A	C	8	0.0	0.0	0.0	0.0	0.0

Table 5 Untransformed data (2 NA)

Effect	Univariate results for each DV (SS data for analysis) Sigma-restricted parameterization Effective hypothesis decomposition				
	Degr. of freedom	2NA propn SS	2NA propn MS	2NA propn F	2NA propn p
Intercept	1	0.096701	0.096701	4.510028	0.037829
Animal	1	0.080345	0.080345	3.747212	0.057611
Location	1	0.018718	0.018718	0.872982	0.353876
Treatment	1	0.018718	0.018718	0.872982	0.353876
Animal*Location	1	0.011946	0.011946	0.557149	0.458326
Animal*Treatment	1	0.011946	0.011946	0.557149	0.458326
Location*Treatment	1	0.096701	0.096701	4.510028	0.037829
Animal*Location*Treatment	1	0.080345	0.080345	3.747212	0.057611
Error	60	1.286482	0.021441		
Total	67	1.80902			

Table 6 Ranked data (2 NA)

Effect	Univariate results for each DV (SS data for analysis) Sigma-restricted parameterization Effective hypothesis decomposition				
	Degr. of freedom	2 NA rank SS	2 NA rank MS	2 NA rank F	2 NA rank p
Intercept	1	54565.1	54565.1	555.0217	0
Animal	1	518.49	518.49	5.2739	0.025154
Location	1	228.73	228.73	2.3266	0.132432
Treatment	1	228.73	228.73	2.3266	0.132432
Animal*Location	1	111.01	111.01	1.1292	0.292213
Animal*Treatment	1	111.01	111.01	1.1292	0.292213
Location*Treatment	1	748.47	748.47	7.6132	0.007671
Animal*Location*Treatment	1	518.49	518.49	5.2739	0.025154
Error	60	5898.7	98.31		
Total	67	9942.5			

Figure 2 2 NA

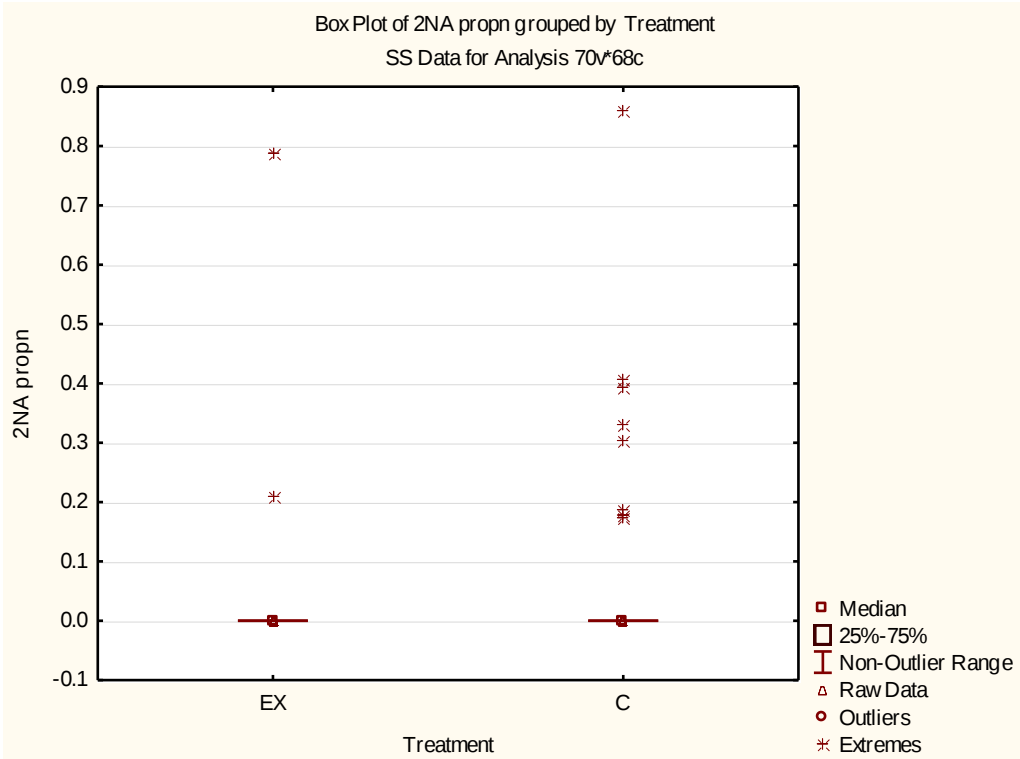


Figure 3 2 NA

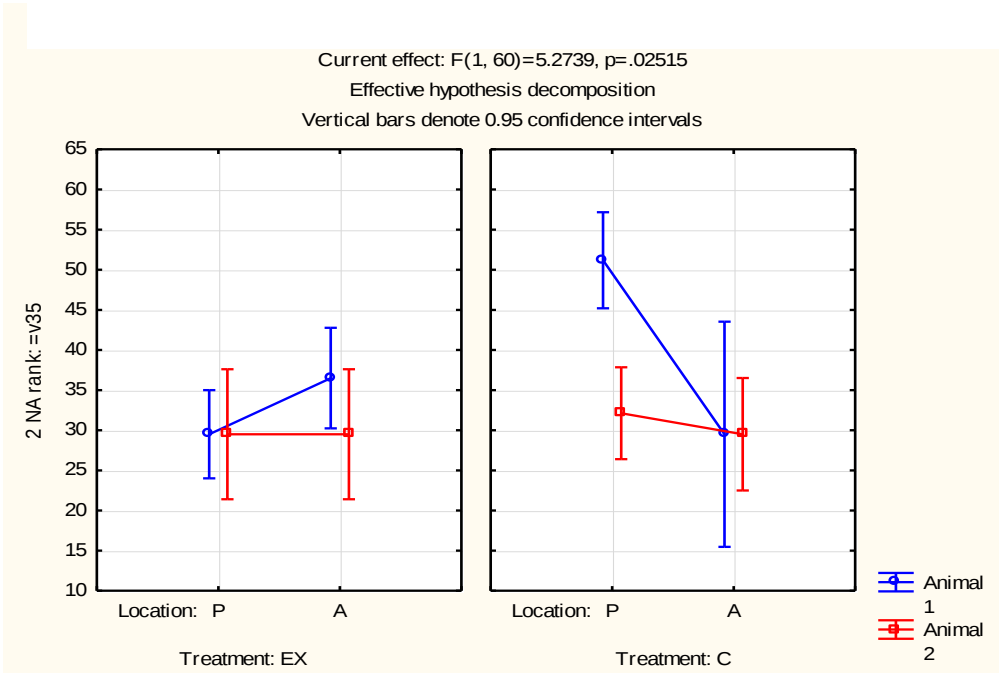


Table 7 (region 3 NA)

3 NA									
Effect	Level of factor	Level of factor	Level of factor	n°	1 NA propn Mean in %	1 NA propn Std.Dev. in %	1 NA propn Std.Err in %	1 NA propn -95 %	1 NA propn 95 %
Total				68	8.8	24.6	3.0	2.9	14.8
Animal	1			36	9.4	26.5	4.4	0.4	18.4
Animal	2			32	8.2	22.7	4.0	0.0	16.4
Location	P			42	7.9	23.8	3.7	0.5	15.3
Location	A			26	10.3	26.3	5.2	-0.3	20.9
Treatment	Ex			35	6.9	22.3	3.8	-0.8	14.6
Treatment	C			33	10.9	27.1	4.7	1.3	20.5
Animal*Location	1	P		24	4.6	19.7	4.0	-3.8	12.9
Animal*Location	1	A		12	19.0	35.8	10.3	-3.7	41.7
Animal*Location	2	P		18	12.3	28.4	6.7	-1.8	26.5
Animal*Location	2	A		14	2.9	10.7	2.9	-3.3	9.1
Animal*Treatment	1	Ex		23	10.5	27.0	5.6	-1.2	22.2
Animal*Treatment	1	C		13	7.4	26.7	7.4	-8.7	23.5
Animal*Treatment	2	Ex		12	0.0	0.0	0.0	0.0	0.0
Animal*Treatment	2	C		20	13.1	27.8	6.2	0.1	26.1
Location*Treatment	P	Ex		19	0.7	3.1	0.7	-0.8	2.2
Location*Treatment	P	C		23	13.8	31.1	6.5	0.4	27.3
Location*Treatment	A	Ex		16	14.3	31.8	7.9	-2.7	31.2
Location*Treatment	A	C		10	4.0	12.7	4.0	-5.1	13.1
Animal*Location*Treatment	1	P	Ex	13	1.0	3.7	1.0	-1.2	3.3
Animal*Location*Treatment	1	P	C	11	8.7	29.0	8.7	-10.7	28.2
Animal*Location*Treatment	1	A	Ex	10	22.8	38.3	12.1	-4.6	50.2
Animal*Location*Treatment	1	A	C	2	0.0	0.0	0.0	0.0	0.0
Animal*Location*Treatment	2	P	Ex	6	0.0	0.0	0.0	0.0	0.0
Animal*Location*Treatment	2	P	C	12	18.5	33.5	9.7	-2.8	39.8
Animal*Location*Treatment	2	A	Ex	6	0.0	0.0	0.0	0.0	0.0
Animal*Location*Treatment	2	A	C	8	5.0	14.2	5.0	-6.9	16.9

Table 8 Untransformed data (3 NA)

Effect	Univariate Results for Each DV (SS Data for Analysis)				
	Sigma-restricted parameterization Effective hypothesis decomposition				
	Degr. of freedom	3NA propn SS	3NA propn MS	3NA propn F	3NA propn p
Intercept	1	0.240582	0.240582	4.060486	0.048385
Animal	1	0.00631	0.00631	0.106493	0.745308
Location	1	0.000014	0.000014	0.000229	0.987969
Treatment	1	0.005392	0.005392	0.091006	0.763945
Animal*Location	1	0.05369	0.05369	0.906157	0.344955
Animal*Treatment	1	0.113992	0.113992	1.923933	0.170555
Location*Treatment	1	0.147898	0.147898	2.496175	0.119381
Animal*Location*Treatment	1	0.022254	0.022254	0.375602	0.542282
Error	60	3.554979	0.05925		
Total	67	4.062732			

Table 9 Ranked data (3 NA)

Effect	Univariate results for each DV (SS data for analysis)				
	Sigma-restricted parameterization Effective hypothesis decomposition				
	Degr. of freedom	3 NA rank SS	3 NA rank MS	3 NA rank F	3 NA rank p
Intercept	1	54852.01	54852.01	384.0337	0
Animal	1	36.19	36.19	0.2533	0.616573
Location	1	7.18	7.18	0.0503	0.823355
Treatment	1	0.01	0.01	0.0001	0.993241
Animal*Location	1	118.2	118.2	0.8276	0.366615
Animal*Treatment	1	477.61	477.61	3.3439	0.072426
Location*Treatment	1	288.63	288.63	2.0208	0.160334
Animal*Location*Treatment	1	77.38	77.38	0.5418	0.464568
Error	60	8569.87	142.83		
Total	67	9942.5			

Figure 4 3 NA

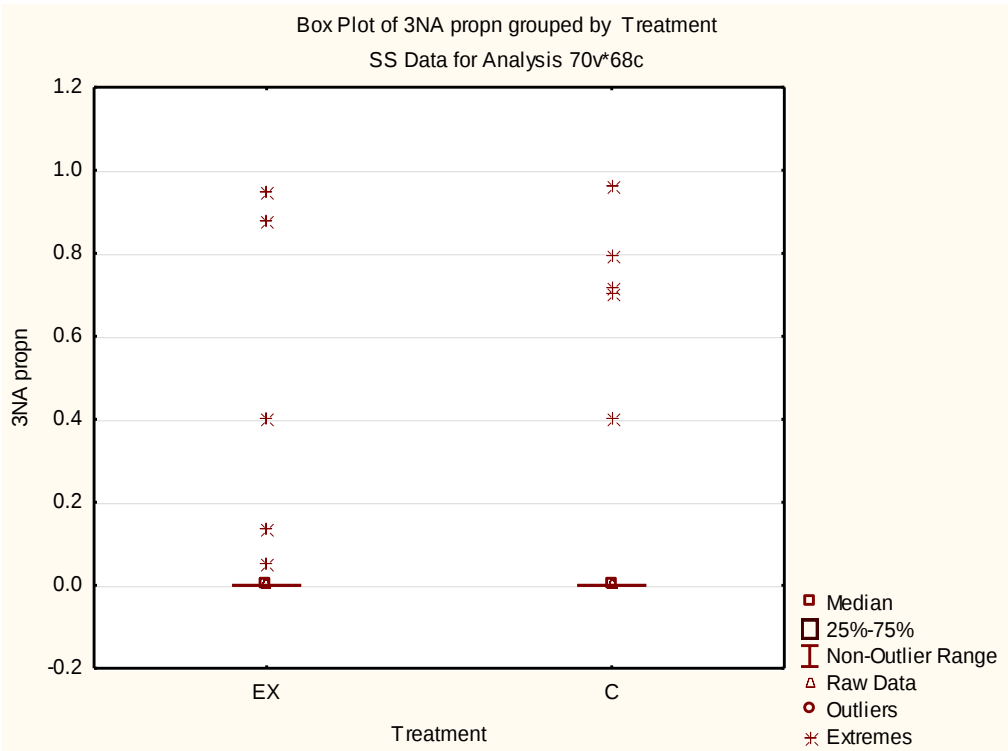


Table 10 (region 4 NA)

4 NA									
Effect	Level of factor	Level of factor	Level of factor	n°	1 NA propn Mean in %	1 NA propn Std.Dev. in %	1 NA propn Std.Err in %	1 NA propn - 95 %	1 NA propn 95 %
Total				68	7.9	21.9	2.7	2.6	13.2
Animal	1			36	14.9	28.5	4.7	5.3	24.5
Animal	2			32	0.0	0.0	0.0	0.0	0.0
Location	P			42	3.8	15.2	2.3	-0.9	8.6
Location	A			26	14.4	28.9	5.7	2.7	26.1
Treatment	Ex			35	10.0	24.3	4.1	1.7	18.4
Treatment	C			33	5.6	19.1	3.3	-1.2	12.4
Animal*Location	1	P		24	6.7	19.8	4.0	-1.6	15.1
Animal*Location	1	A		12	31.2	36.4	10.5	8.1	54.4
Animal*Location	2	P		18	0.0	0.0	0.0	0.0	0.0
Animal*Location	2	A		14	0.0	0.0	0.0	0.0	0.0
Animal*Treatment	1	Ex		23	15.2	28.8	6.0	2.8	27.7
Animal*Treatment	1	C		13	14.3	29.0	8.1	-3.3	31.8
Animal*Treatment	2	Ex		12	0.0	0.0	0.0	0.0	0.0
Animal*Treatment	2	C		20	0.0	0.0	0.0	0.0	0.0
Location*Treatment	P	Ex		19	2.3	10.1	2.3	-2.6	7.2
Location*Treatment	P	C		23	5.1	18.5	3.9	-2.9	13.1
Location*Treatment	A	Ex		16	19.1	32.4	8.1	1.9	36.4
Location*Treatment	A	C		10	6.8	21.6	6.8	-8.6	22.3
Animal*Location*Treatment	1	P	Ex	13	3.4	12.3	3.4	-4.0	10.8
Animal*Location*Treatment	1	P	C	11	10.7	26.2	7.9	-6.9	28.3
Animal*Location*Treatment	1	A	Ex	10	30.6	36.9	11.7	4.3	57.0
Animal*Location*Treatment	1	A	C	2	34.2	48.3	34.2	-399.8	468.1
Animal*Location*Treatment	2	P	Ex	6	0.0	0.0	0.0	0.0	0.0
Animal*Location*Treatment	2	P	C	12	0.0	0.0	0.0	0.0	0.0
Animal*Location*Treatment	2	A	Ex	6	0.0	0.0	0.0	0.0	0.0
Animal*Location*Treatment	2	A	C	8	0.0	0.0	0.0	0.0	0.0

Table 11 Untransformed data (4 NA)

Effect	Univariate results for each DV (SS data for analysis) Sigma-restricted parameterization Effective hypothesis decomposition				
	Degr. of Freedom	4NA propn SS	4NA propn MS	4NA propn F	4NA propn p
Intercept	1	0.475055	0.475055	12.27505	0.000874
Animal	1	0.475055	0.475055	12.27505	0.000874
Location	1	0.196412	0.196412	5.07513	0.027941
Treatment	1	0.008888	0.008888	0.22966	0.63352
Animal*Location	1	0.196412	0.196412	5.07513	0.027941
Animal*Treatment	1	0.008888	0.008888	0.22966	0.63352
Location*Treatment	1	0.001079	0.001079	0.02787	0.867981
Animal*Location*Treatment	1	0.001079	0.001079	0.02787	0.867981
Error	60	2.322049	0.038701		
Total	67	3.211462			

Table 12 Ranked data (4 NA)

Effect	Univariate results for each DV (SS data for analysis) Sigma-restricted parameterization Effective hypothesis decomposition				
	Degr. of Freedom	4 NA rank SS	4 NA rank MS	4 NA rank F	4 NA rank p
Intercept	1	61255.29	61255.29	569.1665	0
Animal	1	1426.49	1426.49	13.2546	0.000568
Location	1	507.52	507.52	4.7157	0.033853
Treatment	1	13.11	13.11	0.1218	0.728298
Animal*Location	1	507.52	507.52	4.7157	0.033853
Animal*Treatment	1	13.11	13.11	0.1218	0.728298
Location*Treatment	1	7.55	7.55	0.0701	0.792085
Animal*Location*Treatment	1	7.55	7.55	0.0701	0.792085
Error	60	6457.37	107.62		
Total	67	9087			

Figure 5 4 NA

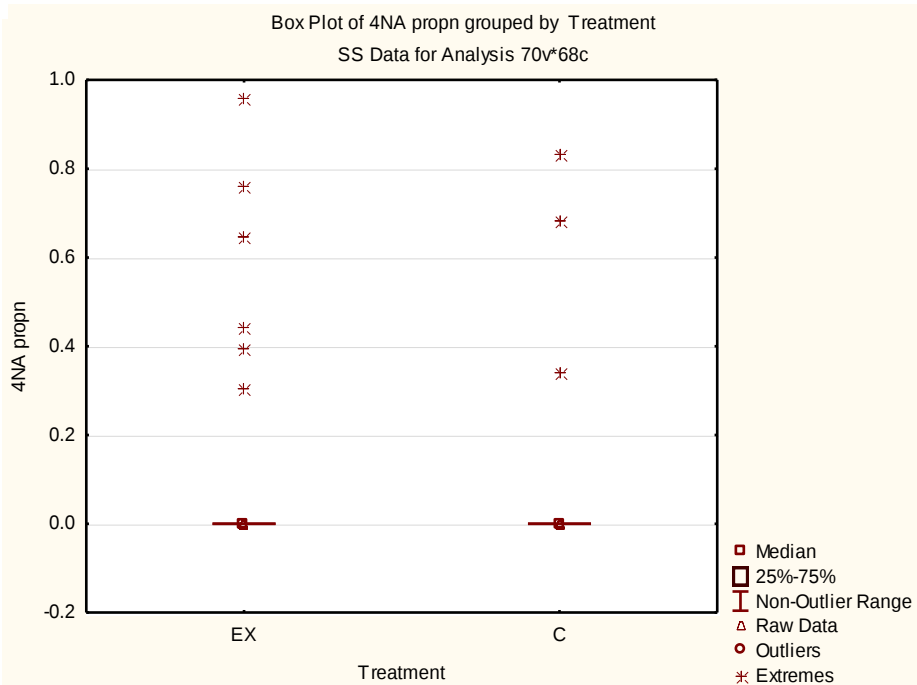


Figure 5 4 NA

