



CHARITÉ 1710-2010
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Evaluation of postnatal anomalies

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Aim

Objectives:

- Evaluation of permanence of anomalies for their classification
- Presentation of exemplary data on skeletal anomalies with suggestions for classification
- Perspective for future approach

Definition of malformation

“Malformation is a permanent structural change that is likely to adversely affect the survival or health of the species under investigation”

[Chahoud et al., 1998, Reprod Toxicol, 13(1): 77-82].

Evaluation of permanence of anomalies for their classification

- The permanence of anomalies can only be evaluated in postnatal studies
- Up to today there is still a lack of data from postnatal evaluations of skeletal anomalies

Presentation of exemplary data on skeletal anomalies with suggestions for classification

Methods:

- Substance: 5-Fluoro-2'-deoxyuridine (FUDR), well-characterized teratogenic agent
- Animals: pregnant Wistar rats
- Treatment: single gavage on GD day 11
- Evaluation of vertebral anomalies on GD 21, PND 7 and PND 21
- Staining of cartilage and bone (Double staining)

Overview examined vertebrae

Day of Examination	GD 21		PND 7		PND 21	
Group	FUDR	Control	FUDR	Control	FUDR	Control
Number of offspring	233	117	308	145	274	86
Number of vertebrae*						
Cervical vertebrae	1631	819	2156	1015	1918	602
Thoracic vertebrae	3029	1521	4004	1885	3562	1118
Lumbar vertebrae	1398	702	1848	870	1644	516
Sacral vertebrae	932	468	1232	580	1096	344
Total	6990	3510	9240	4350	8220	2580

Results: cervical vertebrae

Treatment: FUDR

Region	Anomaly	GD 21			PND 7			PND 21		
		Examined Vertebrae (n)	Vertebrae with Anomaly (n)	Vertebrae with Anomaly (%)	Examined Vertebrae (n)	Vertebrae with Anomaly (n)	Vertebrae with Anomaly (%)	Examined Vertebrae (n)	Vertebrae with Anomaly (n)	Vertebrae with Anomaly (%)
Cervical centrum	unossified	1631	1387	85.04	2156	6	0.28	1918	13	0.68
	dumbbell-shaped	244	6	2.46	2150	9	0.42	1905	24	1.26
Proc.odontoideus	unossified	233	220	94.42	308	0	0.00	274	0	0.00
	asymmetric ossification	13	0	0.00	308	106	34.42	274	49	17.88
	bipartite ossification	13	0	0.00	308	174	56.49	274	164	59.85
	dumbbell-shaped	13	0	0.00	308	96	31.17	274	70	25.55

* p < 0.05 one-tailed Yates' continuity corrected chi-square test

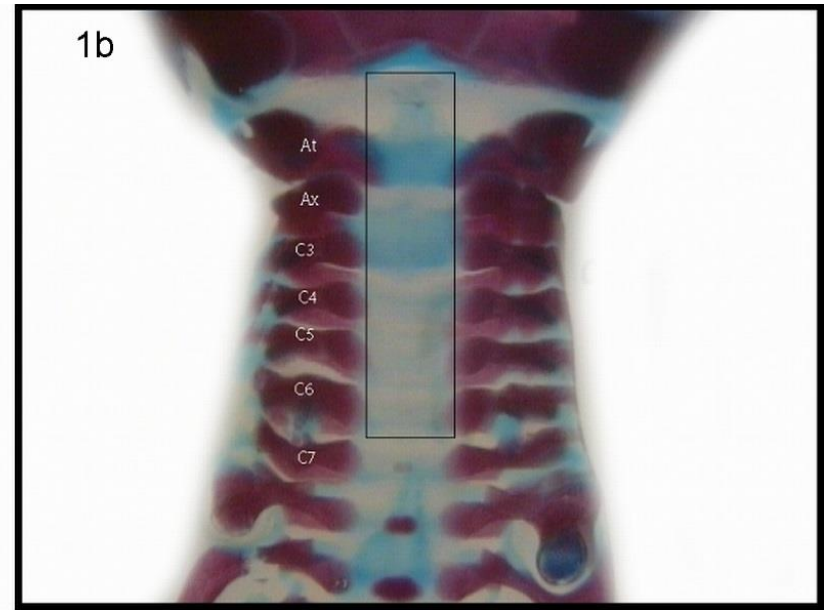
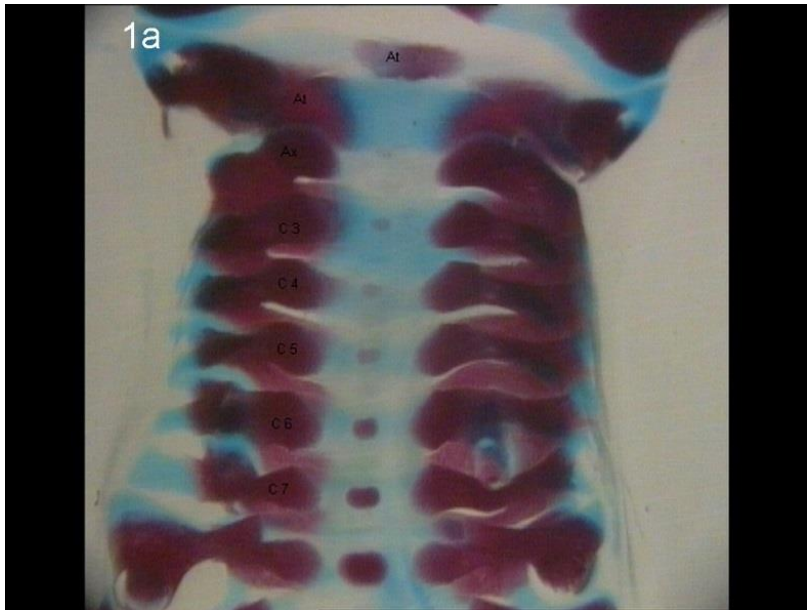


Fig 1 a-b Grade of ossification in cervical vertebrae on GD 21. Fig. 1a demonstrates ossified cervical centers with different grades of ossification. Fig. 1b shows unossified cervical centers. At = atlas, Ax = axis, C3-C7 = cervical vertebrae. The box indicates the regions without ossification centers.

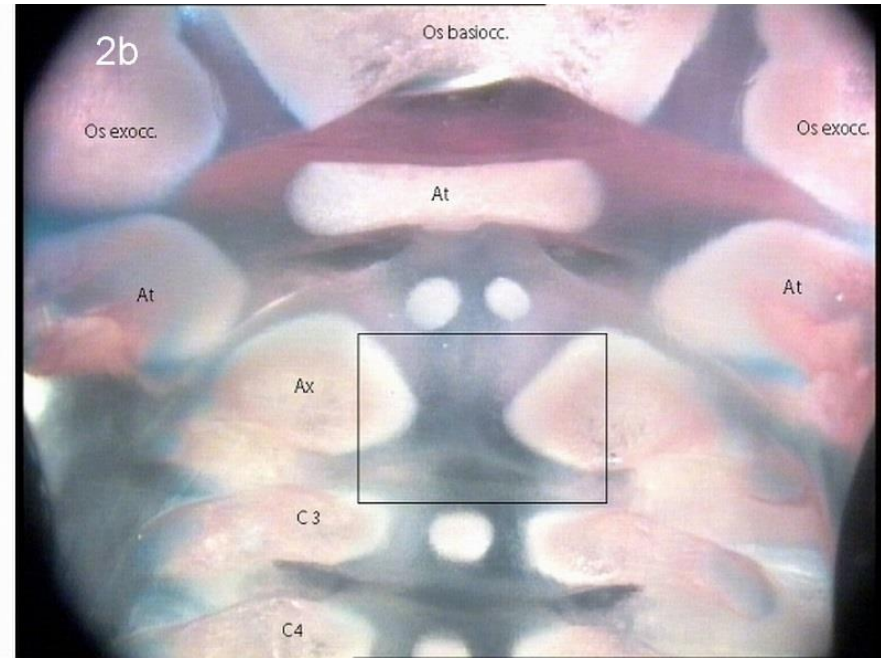
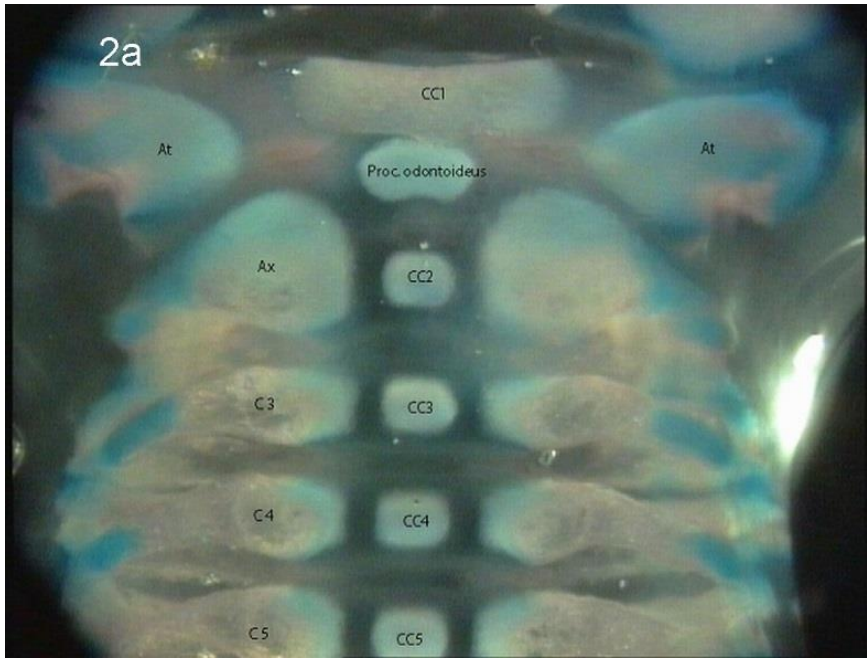


Fig 2 a-b Grade of ossification in cervical vertebrae on PND7. Fig. 2a illustrates ossified cervical centers. Fig. 2b depicts absent cervical center on CC2. At = atlas, Ax = axis, C3-C4 = cervical vertebrae, CC1-CC5 = cervical centrum, Box indicates the region where CC2 is missing

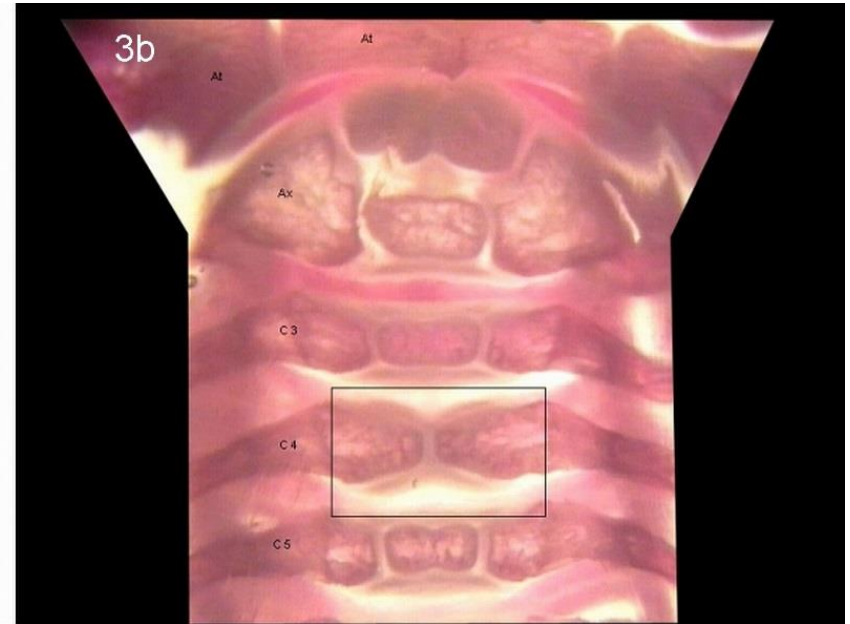
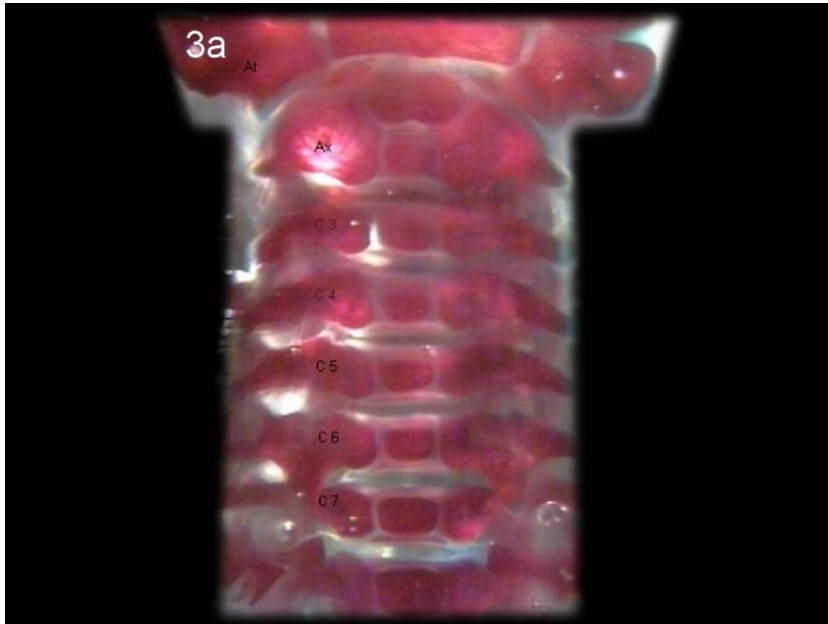


Fig 3. a-b Grade of ossification in cervical vertebrae on PND21. Fig. 3a shows ossified cervical centers. Fig. 3b The box indicates the absent cervical center on CC4. At = atlas, Ax = axis, C3-C7 = cervical vertebrae.

Results: thoracic vertebrae

Treatment: FUDR

Region	Anomaly	GD 21			PND 7			PND 21		
		Examined Vertebrae (n)	Vertebrae with Anomaly (n)	Vertebrae with Anomaly (%)	Examined Vertebrae (n)	Vertebrae with Anomaly (n)	Vertebrae with Anomaly (%)	Examined Vertebrae (n)	Vertebrae with Anomaly (n)	Vertebrae with Anomaly (%)
Thoracic centrum	unossified	3029	77	2.54	4004	0	0.00	3562	0	0.00
	asymmetric ossification	2952	281	9.52	4004	1	0.02	3562	0	0.00
	bipartite ossification	2952	1347	45.63	4004	207	5.17	3562	107	3.00
	dumbbell-shaped	2952	130	4.40	4004	57	1.42	3562	74	2.08*
	hemicentric	2952	37	1.25	4004	0	0.00	3562	0	0.00
	misshapen	2952	4	0.14	4004	0	0.00	3562	3	0.08

* p < 0.05 one-tailed Yates' continuity corrected chi-square test

Results: lumbar vertebrae

Treatment: FUDR

Region	Anomaly	GD 21			PND 7			PND 21		
		Examined Vertebrae (n)	Vertebrae with Anomaly (n)	Vertebrae with Anomaly (%)	Examined Vertebrae (n)	Vertebrae with Anomaly (n)	Vertebrae with Anomaly (%)	Examined Vertebrae (n)	Vertebrae with Anomaly (n)	Vertebrae with Anomaly (%)
Lumbar centrum	unossified	1398	68	4.86	1848	0	0.00	1644	0	0.00
	asymmetric ossification	1330	115	8.65	1848	7	0.38	1644	0	0.00
	misshapen	1330	340	25.56	1848	44	2.38	1644	29	1.76

Results: sacral vertebrae

Treatment: FUDR

Region	Anomaly	GD 21			PND 7			PND 21		
		Examined Vertebrae (n)	Vertebrae with Anomaly (n)	Vertebrae with Anomaly (%)	Examined Vertebrae (n)	Vertebrae with Anomaly (n)	Vertebrae with Anomaly (%)	Examined Vertebrae (n)	Vertebrae with Anomaly (n)	Vertebrae with Anomaly (%)
Sacral centrum	unossified	932	155	16.63	1232	0	0.00	1096	1	0.09
	asymmetric ossification	777	20	2.57	1232	3	0.24	1095	1	0.09
	bipartite ossification	777	42	5.41	1232	22	1.79	1095	10	0.91
	hemicentric	777	55	7.08	1232	4	0.32	1095	0	0.00
	misshapen	777	57	7.34	1232	6	0.49	1095	0	0.00
Sacral arch	misshapen	932	81	8.69	1232	2	0.16	1096	0	0.00

* p < 0.05 one-tailed Yates' continuity corrected chi-square test

Suggestions for classification Variation

Variations: anomalies did not persist up to PND21

- Unossified
- Asymmetric ossification
- Bipartite ossification
- Hemicentric
-
- Misshapen: in thoracic, lumbar and sacral region

Suggestions for classification Malformation

Malformation: anomalies were persistent up to PND 21

- Cervical vertebra centrum dumbbell-shaped
- Lumbar centrum supernumerary
sinister/dexter/sinister+dexter
- Misshapen: in cervical region?

Perspective for future approach

- Currently no guidelines existent for studies of postnatal fate of anomalies
- Guidelines are needed for well-founded classification
- Inclusion in existing guidelines? E.g. Extended One-Generation study?
- No additional animals should be used
- Applicable and reproducible in every lab
- Timepoint/age at evaluation?

Thank you for your attention!