SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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VERSION 2.0 (DECEMBER 2014)

Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	The long-term consequences of disbudding in cattle	
2.	Authors (names, affiliations, contributions)	Vivian Goerlich[1], Josefine Stuff[2], Ellen Meijer[1], Marijke Algra[1], Saskia Arndt[1] [1]Department of Population Health Sciences, Division of Animals in Science and Society, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands [2]Precision Livestock Farming, Faculty of Agricultural Sciences and Landscape Architecture, Osnabrueck University of Applied Sciences, Emsweg 3, 49090 Osnabrueck, Germany Contributions: VG and MA conceived the presented idea. SA and VG acquired funding from the Office for Risk Assessment & Research, the Netherlands. VG, MA, and EM defined the literature search and exclusion criteria.	
3.	Other contributors (names, affiliations, contributions)	students	
4.	Contact person + e-mail address	Vivian Goerlich, v.c.goerlich@uu.nl	
5.	Funding sources/sponsors	Office for Risk Assessment & Research, the Netherlands (BuRO)	
6.	Conflicts of interest	None	
7.	Date and location of protocol registration	https://syreaf.org/protocols/	
8.	Registration number (if applicable)		
9.	Stage of review at time of registration	TiAb Screening	
	B. Objectives		
	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	Most commercially kept (dairy) calves are disbudded at an early age, thus the horn buds are removed, preventing the growth of horns. While the most common method is using a hot iron, the use of caustic paste is increasing. This procedure is known to cause acute pain and distress, the long-term consequences, however, are less	

		investigated. With this synthesis of evidence, we aim to analysis the current knowledge on the effects of disbudding, and the absence of horns, on indicators of welfare.	
	Research question		
11.	Specify the disease/health problem of interest	N/A, this is a veterinary science review.	
12.	Specify the population/species studied	Cattle (dairy, beef)	
13.	Specify the intervention/exposure	Disbudding (removal of horn buds) by any method, e.g. hot iron, caustic paste.	
14.	Specify the control population	sham; placebo; analgesia and/or anesthesia; alternative method of disbudding	
15.	Specify the outcome measures	Measured minimum >=6 days post-disbudding: • Wound healing • Physiological indicators of stress (CORT, etc) • Physical condition; health • Behaviour • Pain indicators (other than behaviour)	
16.	State your research question (based on items 11-15)	What are the long-term consequences of horn removal on indicators of welfare in cattle?	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	X MEDLINE via PubMed ☐ Web of Science X SCOPUS ☐ EMBASE X Other, namely: CABi ☐ Specific journal(s), namely:	
18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20, 21])	When available, please add a supplementary file containing your search strategy: see end of the protocol	
19.	Identify other sources for study identification	X Reference lists of included studies X Books X Reference lists of relevant reviews ☐ Conference proceedings, namely: ☐ Contacting authors/ organisations, namely: ☐ Other, namely: for the included studies, use Google Scholar's "cited by" feature	
20.	Define search strategy for these other sources	Reference lists will be screened based on title for potentially relevant additional studies not yet retrieved by the comprehensive search. Potentially relevant studies will then undergo title and abstract screening and subsequent full-text screening as described below.	
	Study selection		

21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	Screening in two phases: 1) screening for eligibility based on title and abstract and 2) full-text screening for final inclusion.	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	Each record will be screened by two reviewers. Discrepancies will be resolved by discussion between the disagreeing reviewers until a consensus is reached. If no consensus is reached, a third reviewer is asked for a final decision.	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	• Wound healing Observational studies are **included**. • Physiological indicators of stress (CORT, etc) • Physical condition; health • Behaviour • Pain indicators (other than behaviour) **Inclusion criteria**: experimental studies including at least two of the following experimental groups: sham; placebo; analgesia and/or anaesthesia; alternative method of disbudding (within-subjects baseline or between subjects). **Exclusion criteria**: experimental studies with no appropriate comparison	
24.	Type of animals/population (*e.g.* age, gender, disease model)	**Inclusion** criteria: cattle **Exclusion** criteria: studies in humans, cells or other animal species, in silico studies, ex vivo studies.	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	**Inclusion** criteria: Disbudding (any method) **Exclusion** criteria: Dehorning (removal of grown horns), genetic selection (e.g., breeding for polledness)	
26.	Outcome measures	**Inclusion** criteria: Any outcome measure with a clear relevance to one of the following themes: • Wound healing • Physiological indicators of stress (CORT, etc) • Physical condition; health • Behaviour • Pain indicators (other than behaviour) **Exclusion** criteria: no relevant outcomes reported	
27.	Language restrictions	**Inclusion** criteria: any language	

		Exclusion criteria: none* * For non-English references, an attempt is made to translate the article either by the authors, native speakers in our network, or using AI powered translation tools. If enough of the article can be translated to extract all necessary information, the article will be included. Otherwise, it will be excluded, but listed as (potentially) relevant.	
28.	Publication date restrictions	**Inclusion** criteria: any **Exclusion** criteria: none	
29.	Other	**Inclusion** criteria: full-length research articles describing unique primary data. **Exclusion** criteria: records without primary data (reviews and books), conference abstracts/posters without a full description of methods or data, datasets published in duplicate; stakeholder surveys	
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase: TiAb screening - **Not in cattle**: about a species other than cattle - **Not disbudding**: about another procedure than disbudding (e.g. dehorning or breeding for polledness) - **Survey** - **No primary data** (reviews and books), conference abstracts/posters without full description of methods and data - **No welfare** consequences measured If wound healing is not investigated: **Absence of comparison** group (sham; placebo; analgesia and/or anesthesia; alternative disbudding method). Observational studies on wound healing are included. Selection phase: Full text screening - **No full text** available (after checking with library) - **Not in English** and not translatable using translate AI - **Not in cattle**: about a species other than cattle - **Survey** - **No primary data** (reviews and books), conference abstracts/posters without full description of methods and data - **Not disbudding**: about another procedure than disbudding (e.g. dehorning or breeding for polledness)	

		- **No details on intervention**: e.g. it is not clear what method of disbudding was used) - **No welfare consequences** measured - If wound healing is not investigated: **Absence of comparison** group (sham; placebo; analgesia and/or anesthesia; alternative disbudding method). Observational studies on wound healing are included. - **Not long-term** (study duration <= 6 days)	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	• Authors *(chr)* • Year *(num)* • Title *(chr)* • Journal *(chr)* • Issue *(num)* • Page numbers *(num)* • Language *(chr)* • DOI *(chr)*	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	• Number of animals - Treatment • Number of animals – Control • Statistical n – Treatment (in case of pooled samples) • Statistical n – Control (in case of pooled samples) • Study design/type of control (within-subject, a between-subject, combined design, observational)	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	• Strain/breed/genetic line • Commercial purpose (e.g., dairy or meat production) • Sex (m / f / mixed) • Housing	

34.	Intervention characteristics (*e.g.* intervention, timing, duration)	• Disbudding Method
		  ○ Hot iron
		  ○ Caustic paste
		  ○ Scoop
		  ○ Other (specify next column)
		  ○ Unsure
		  ○ Not specified
		• Specify disbudding method (free text)
		• Age at 1st disbudding (days or mean)
		  ○ Intervention group
		  ○ sham disbud
		  ○ placebo disbud
		  ○ analgesia &/or anaesthesia disbud
		  ○ Other (specify intervention free txt)
		• Intervention group free text (drugs & shaving)
		• Wound treatment?	

		• Additional interventions? (e.g., housing) • Age at end of data collection (in days or mean) (minimum=>6 days)	
35.	Outcome measures	List any outcome measure with a clear relevance to • Wound healing • Physiological indicators of stress (CORT, etc) • Physical condition; health • Behaviour • Pain indicators	
36.	Other (*e.g.* drop-outs)	Not applicable	
	Assessment risk of bias (internal validity) or study quality		
37.	Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved	a) Two reviewers will independently assess the risk of bias b) Discrepancies will be resolved by discussion of the two reviewers who performed the RoB. If no consensus can be reached, a third reviewer is asked for a final decision	
38.	Define criteria to assess (a) the internal validity of included studies (*e.g.* selection, performance, detection and attrition bias) and/or (b) other study quality measures (*e.g.* reporting quality, power)	X By use of SYRCLE's Risk of Bias tool[4] ☐ By use of SYRCLE's Risk of Bias tool, adapted as follows: ☐ By use of CAMARADES' study quality checklist, e.g [22] ☐ By use of CAMARADES' study quality checklist, adapted as follows: ☐ Other criteria, namely:	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	Whether a study measured a certain outcome within the categories (Wound healing; Physiological indicators of stress; Physical condition; health, Behaviour, Pain indicators): yes/ no → dichotomous Quantitative data will be extracted for studies that will be included in a meta-analysis (see below). For continuous outcomes, we will extract the mean, SD and n in all control and experimental groups. The SD may be recalculated from the SEM. For dichotomous data the incidences out of the total n in all control and experimental group will be extracted.	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	Data will be extracted from text and tables. If only reported graphically, we will use digital ruler software to extract data from graphs. In case of missing data or data reported as ranges (e.g., "we used 20-24 animals per group"), we will make a conservative estimate of the data, or exclude the article from the analysis if no conservative estimate can be made.	

41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	Data will be extracted by one reviewer and checked by a second. Discrepancies will be resolved by discussion until a consensus is reached. If needed, a third reviewer will be asked for a final decision	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	We will perform a descriptive summary of the study characteristics (breed, disbudding method; nr of studies that measured outcome parameters). In addition, we will perform a meta-analysis when deemed feasible in terms of the quantity and heterogeneity of the evidence (see below)	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	For each of the outcome measure categories defined above, we will assess a) whether enough evidence has been reported to perform meta-analysis with sufficient power (i.e. k >= 3) and b) whether the outcomes reported are homogeneous enough in terms of the type of outcome and its interpretation for welfare for sensible pooling of the result. For subgroup analysis, a minimum of 3 studies in at least two of the subgrouping categories is needed to perform the analysis.	
	If a meta-analysis seems feasible/sensible, specify (for each outcome measure):		
44.	The effect measure to be used (*e.g.* mean difference, standardized mean difference, risk ratio, odds ratio)	Standardised mean difference for continuous outcomes (since we expect the unit of measurement to differ between outcomes) Risk ratio for dichotomous outcomes (likely a minority)	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Random effects model to account for expected differences between studies	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	I^2 statistic for overall analyses and the adjusted R^2 statistic for subgroup analysis	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	• Breed • Age at disbudding • Age at measurement • Disbudding method • Intervention group (sham control, placebo control, analgesia and/or anaesthesia, wound treatment) • Additional intervention (e.g., wound treatment, housing, nutrition, etc)	
48.	Any sensitivity analyses you propose to perform	Not planned	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	If a study compares the same control group to multiple intervention groups, the number of control animals included in our meta-analysis will be corrected by dividing the number of control animals by the number of comparisons made. To correct for multiple subgroup analyses, we will adjust the significance level for subgroup analyses using the Bonferroni-Holmes correction.	

50.	The method for assessment of publication bias	A Risk of Bias (RoB) assessment using SYRCLE's RoB tool will be performed. If a meta-analysis is performed, a funnel plot will be created if feasible (k > 10) and publication bias will be assessed using Egger's regression test (if k > 10).	

Final approval by (names, affiliations):

Ellen Meijer1

Josefine Stuff2,

Saskia Arndt1,

Vivian Goerlich1

[1]Department of Population Health Sciences, Division of Animals in Science and Society, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

[2]Precision Livestock Farming, Faculty of Agricultural Sciences and Landscape Architecture, Osnabrueck University of Applied Sciences, Emsweg 3, 49090 Osnabrueck, Germany

Date: 31-08-2026

Search string:

Pubmed Title/Abstract
(disbud*[TiAB] OR dehorn*[TiAB] OR cauterization[TiAB] OR cauterisation[TiAB] OR cautery[TiAB] OR caustic paste[TiAB] OR chemical disbudding[TiAB] OR chemical cauterization[TiAB] OR chemical cauterisation[TiAB])

AND
(cattle [MeSH Terms] OR cattle[TiAB] OR cow[TiAB] OR cows[TiAB] OR bovin*[TiAB] OR calf[TiAB] OR calves[TiAB] OR "bos taurus"[TiAB] OR "Dairy Cow"[TiAB] OR "Cow, Dairy"[TiAB] OR "Dairy Cows"[TiAB] OR "Beef Cow" [TiAB] OR "Beef Cows"[TiAB] OR "Cow, Beef"[TiAB])

Scopus TITLE-ABS-KEY
(disbud* OR dehorn* OR cauterization OR cauterisation OR cautery OR caustic paste OR chemical disbudding OR chemical cauterization OR chemical cauterisation)

AND
(cattle OR cow OR cows OR bovin* OR calf OR calves OR bos taurus OR Dairy Cow OR Cow, Dairy OR Dairy Cows OR Beef Cow OR Beef Cows OR Cow, Beef)

CAB Digital library ALL FIELDS
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