**Table S1.** Finalized search strategy for included databases.

| Database | Search strategy | Results |
|---|---|---|
| PubMed | (((((("Eye"[MeSH Terms])) OR (("Eye Diseases"[MeSH Terms]))) AND ((("ex vivo")) OR (("in vivo")))) AND ((((("dosage form design")) OR (("drug delivery"))) OR (("Therapeutics"[MeSH Terms]) OR ("Drug-Related Side Effects and Adverse Reactions"[MeSH Terms])))) AND (((((("Ultrasonography"[MeSH Terms]) OR (ultrasound)) OR ("focused ultrasound")) OR ("MRI-guided ultrasound")) OR ("MR-guided focused ultrasound")) | 249 |
| Embase | (eye* OR 'eye disease' OR 'retinal disease')/br AND (('in vivo' OR 'ex vivo'):ti,ab,kw) AND ((therapy OR therapeutics OR 'drug dosage form' OR 'drug delivery method' OR 'adverse drug reactions' OR 'instillation')/br) AND ((ultrasonography OR ultrasound OR 'MRI-guided ultrasound' OR 'MR-guided focused ultrasound')/exp) | 293 |
| SCOPUS | (TITLE-ABS-KEY(("eye disease*") OR ("eye*") OR ("retina*") OR ("optic nerve") OR ("age-related macular degeneration") OR ("optic nerve disease*") OR ("amd") OR ("posterior eye*")) AND TITLE-ABS-KEY(("in vivo") OR ("ex vivo")) AND TITLE-ABS-KEY(("therap*") OR ("dosage form design") OR ("drug delivery") OR ("adverse drug reaction")) AND TITLE-ABS-KEY((ultrasound) OR (ultrasonography))) | 161 |
| CINAHL | TX (eye* OR "eye diseases" OR retina OR "optic nerve" OR "age-related macular degeneration" OR "optic nerve disease" OR amd OR "posterior eye disease") AND TX ("in vivo" or "ex vivo") AND AB ("therap*" OR "dosage form design" OR "drug delivery" OR "adverse drug reaction") AND TX (ultrasound or ultrasonography) | 134 |

Example search strategy development process:

## History and Search Details

Download

| Search | Actions | Details | Query | Results |
|---|---|---|---|---|
| #20 | ••• | > | Search: (((((("Eye"[MeSH Terms])) OR (("Eye Diseases"[MeSH Terms]))) AND ((("ex vivo")) OR (("in vivo")))) AND ((((("dosage form design")) OR (("drug delivery"))) OR (("Therapeutics"[MeSH Terms]) OR ("Drug-Related Side Effects and Adverse Reactions"[MeSH Terms])))) AND (((((("Ultrasonography"[MeSH Terms]) OR (ultrasound)) OR ("focused ultrasound")) OR ("MRI-guided ultrasound")) OR ("MR-guided focused ultrasound")) | 249 |
| #19 | ••• | > | Search: ((((("Ultrasonography"[MeSH Terms]) OR (ultrasound)) OR ("focused ultrasound")) OR ("MRI-guided ultrasound")) OR ("MR-guided focused ultrasound") | 1,857,123 |
| #18 | ••• | > | Search: "MR-guided focused ultrasound" | 294 |
| #17 | ••• | > | Search: "MRI-guided ultrasound" | 1 |
| #16 | ••• | > | Search: "focused ultrasound" | 7,595 |
| #15 | ••• | > | Search: ultrasound | 1,857,123 |
| #14 | ••• | > | Search: "Ultrasonography"[MeSH Terms] | 475,276 |
| #13 | ••• | > | Search: ((("dosage form design")) OR (("drug delivery"))) OR (("Therapeutics"[MeSH Terms]) OR ("Drug-Related Side Effects and Adverse Reactions"[MeSH Terms])) | 5,131,573 |
| #12 | ••• | > | Search: ("Therapeutics"[MeSH Terms]) OR ("Drug-Related Side Effects and Adverse Reactions"[MeSH Terms]) | 5,089,154 |
| #11 | ••• | > | Search: (("dosage form design")) OR (("drug delivery")) | 140,908 |
| #10 | ••• | > | Search: "Drug-Related Side Effects and Adverse Reactions"[MeSH Terms] | 127,297 |
| #9 | ••• | > | Search: "Therapeutics"[MeSH Terms] | 4,995,359 |
| #8 | ••• | > | Search: ("drug delivery") | 140,764 |
| #7 | ••• | > | Search: ("dosage form design") | 199 |
| #6 | ••• | > | Search: (("ex vivo")) OR (("in vivo")) | 1,067,318 |
| #5 | ••• | > | Search: ("ex vivo") | 86,745 |
| #4 | ••• | > | Search: ("in vivo") | 1,010,400 |
| #3 | ••• | > | Search: (("Eye"[MeSH Terms])) OR (("Eye Diseases"[MeSH Terms])) | 794,046 |
| #2 | ••• | > | Search: ("Eye"[MeSH Terms]) | 378,837 |
| #1 | ••• | > | Search: ("Eye Diseases"[MeSH Terms]) | 615,128 |

Showing 1 to 20 of 20 entries

**Table S3:** Arrive 2.0 questionnaire for systematic review.

| # | ARRIVE 2.0 item | Operational question | Answer | | |
|---|---|---|---|---|---|
| | | | Yes | No | N/A |
| **Required set** | | | | | |
| 1 | 1a. Study design | Are the treatment and control groups adequately described and comparable? | | | |
| 2 | 1b. Study design | Are the individual experimental units clearly defined for accurate analysis, without the risk of pseudo-replication of results | | | |
| 3 | 2a. Sample size | Are the exact number of experimental units allocated to each group (Tx or control) specified? Are the total number of animals used specified? | | | |
| 4 | 2b. Sample size | Is it explained how the sample size was decided? Were power calculations used to determine sample size? If not, is there justification for the lack thereof? | | | |
| 5 | 3a. Inclusion and exclusion criteria | Were any criteria for including or excluding animals during the experiment, or data points during the analysis specified? Were they established *a priori*, is there justification for the lack thereof? | | | |
| 6 | 3b. Inclusion and exclusion criteria | For each experimental group, is it reported whether any animals, experimental units, or data points were not included in the analysis? Is it stated if there were no exclusions? | | | |
| 7 | 3c. Inclusion and exclusion criteria | Is the exact n value included in analysis in each group reported? | | | |
| 8 | 4a. Randomisation | Was randomisation used to allocate experimental units to control and treatment groups? | | | |
| 9 | 4b. Randomisation | Was a strategy used to minimise the potential for confounders? E.g. order of treatments, and measurements, animal cage/location | | | |
| 10 | 5. Blinding | Is it reported who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis) | | | |
| 11 | 6a. Outcome measures | Are all the outcome measures explicitly described? | | | |
| 12 | 6b. Outcome measures | In studies using inferential statistics (t test, ANOVA), was the primary outcome measure specified? | | | |
| 13 | 7a. Statistical methods | Are details of the statistical methods used for each analysis included? | | | |
| 14 | 7b. Statistical methods | Were there methods used to assess whether the data met the underlying assumptions of the statistical approach? (e.g. tests for normality to ensure the parametric tests' assumptions were valid) | | | |
| 15 | 8a. Experimental animals | Were species-appropriate details of the animals given? (e.g. species, strain, sex, age and, if relevant, weight) | | | |
| 16 | 8b. Experimental animals | Was further information on the provenance, health, and any previous procedures given to test animals? | | | |
| 17 | 9a. Experimental procedures | Were enough details given for each experimental group, including controls, pertaining to the procedures, to allow others to replicate them? Including: what was done, how was it done and what was used? | | | |
| 18 | 9b. Experimental procedures | Is it reported when and how often experimental procedures and measurements were given? | | | |
| 19 | 9c. Experimental procedures | Is it stated if physiological acclimatisation after a stressful event, such as transport or treatments/handling were allowed before treatment or assessment? (if applicable) | | | |
| 20 | 9d. Experimental procedures | Is it explained why experimental procedures were undertaken in the way they had been completed? | | | |

| | | | | | |
|---|---|---|---|---|---|
| 21 | 10a. Results | Has a summary/descriptive statistic of the results for each experimental group been reported? Including a measure of variability where applicable? | | | |
| 22 | 10b. Results | Has the effect size been reported with a confidence interval? | | | |
| **Recommended set** | | | | | |
| 23 | 11. Abstract | Does the abstract provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings and study conclusions? | | | |
| 24 | 12a. Background | Is there a sufficient scientific background to understand the rationale and context for the study? Is the experimental approach explained? | | | |
| 25 | 12b. Background | Is it explained why the animal was used, or how the animal species and model used addresses the scientific objectives, and, where appropriate, the relevance to human biology? | | | |
| 26 | 13. Objectives | Are the research question and objectives and, where appropriate, the specific hypothesis being tested clearly described? | | | |
| 27 | 14. Ethical statement | Is the ethical review committee or equivalent which approved the use of animals in this study named? Has the use of animals been approved? | | | |
| 28 | 15. Housing and husbandry | Are details of housing and husbandry conditions, including any environmental enrichment, provided? | | | |
| 29 | 16a. Animal care and monitoring | Is it reported whether any interventions or steps were taken in the experimental protocols to reduce pain, suffering and distress? | | | |
| 30 | 16b. Animal care and monitoring | Are expected or unexpected adverse events reported? | | | |
| 31 | 16c. Animal care and monitoring | Are the humane endpoints established for the study? Is it reported what signs were monitored and the frequency of monitoring? | | | |
| 32 | 17a. Interpretation/ scientific implications | Are the results interpreted whilst taking into account the study objectives, hypotheses, current theory and other relevant studies in the literature? | | | |
| 33 | 17b. Interpretation/ scientific implications | Are the study limitations, including potential sources of bias, limitations of the animal model and imprecision with the results commented on? | | | |
| 34 | 18. Generalisability/ translation | Is it commented on as to whether or how the findings of the study are likely to generalise to other species or experimental conditions, including any relevance to human biology? | | | |
| 35 | 19. Protocol registration | Is it stated whether a protocol (including research questions, key design features and analysis plan) was prepared before the study, and if and where this protocol was registered? | | | |
| 36 | 20. Data access | Is a statement provided describing if and where the study data are available? | | | |
| 37 | 21a. Declaration of interests | Are any potential conflicts of interest, including financial and nonfinancial declared? If none exist, this should be stated | | | |
| 38 | 21b. Declaration of interests | Are all funding sources (including grant identifier), and their role in the design, analysis and reporting of the study listed? | | | |

**Table S4. Determination of mean cow vitreal volume**

| | |
|---|---|
| Title | Measurement of cow eye vitreous volume |
| Date of experiment | 08.12.2022 (DD.MM.YYYY) |
| Objective | Determine the average vitreal volume of cow eyes to include in the systematic review |
| Rationale | Currently literature is lacking in information related to the typical volume of the bovine eye vitreous |
| Experimental outline | 1. Collect eyes in DMEM<br>2. Remove aqueous humour<br>3. Remove vitreous humour, centrifuge, and measure volume |
| Materials | 1. Cow eyes<br>2. Pipettes and pipette tips<br>3. Tuberculin syringes<br>4. Centrifuge<br>5. 20ml Measuring cylinder<br>6. DMEM + P/S/L-Glut<br>7. Butterfly imaging ultrasound transducer |
| Method | 1. Day 1<br>  1. Prepare DMEM in biosecurity cabinet<br>  a. Add 5 mL 100X Pen/Strep/L-Glut to each bottle of 500 mL DMEM<br>2. Day 2<br>  1. Collect cow eyes from Teys Australia Pty LTD<br>  a. Collect in DMEM, note which eyes relate to which cows<br>  b. Collect cold, in ice, note time of death and time of collection<br>  2. Remove majority of aqueous humour from anterior segment using tuberculin syringes<br>  3. Cut through cornea and remove remaining aqueous humour<br>  4. Remove lens<br>  5. Remove vitreous humour into 20ml falcon tube<br>  6. Measure weight of vitreous<br>  7. Determine vitreous volume using by applying density of 1.005g/cm$^3$ |

Animal details

| Mob | Body ID | Dentition | Gender | Body Weight (kg) | Bruise | Time of slaughter | Time of collection | Cow ID |
|---|---|---|---|---|---|---|---|---|
| 407 | 312 | 0 | Male | 288.2 | 0 | 08:44 | 09:15 | 1 |
| 407 | 315 | 0 | Male | 292.2 | 0 | 08:46 | 09:16 | 3 |
| 407 | 316 | 0 | Male | 290.6 | 0 | 08:46 | 09:16 | 5 |
| 407 | 317 | 0 | Male | 271.6 | 0 | 08:47 | 09:17 | 7 |
| 407 | 318 | 0 | Male | 292.2 | 0 | 08:47 | 09:17 | 9 |
| 407 | 320 | 2 | Male | 292.8 | 0 | 08:48 | 09:18 | 11 |
| 407 | 321 | 0 | Male | 272.2 | 0 | 08:49 | 09:19 | 13 |
| 407 | 322 | 2 | Male | 295.2 | 0 | 08:50 | 09:19 | 15 |
| 407 | 323 | 0 | Male | 295 | 0 | 08:50 | 09:20 | 17 |
| 407 | 324 | 0 | Male | 275.2 | 0 | 08:51 | 09:20 | 19 |

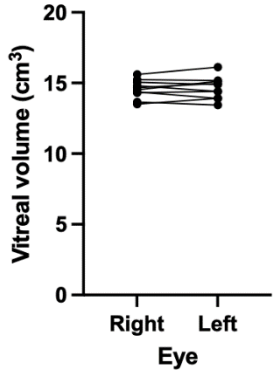
Note, cow age is appraised according to dentition, where 0 = ≤ 18 months, and 2 = 18-30 months

Results

| **Weight measurements (g)** | | |
|---|---|---|
| Eye ID | Left eye | Right eye |
| 1 | 15.055 | 14.742 |
| 2 | 16.198 | 15.68 |
| 3 | 14.96 | 15.126 |
| 4 | 14.483 | 14.374 |
| 5 | 14.487 | 14.882 |
| 6 | 13.974 | 14.452 |

| | | |
|---|---|---|
| 7 | 15.257 | 15.31 |
| 8 | 13.997 | 13.581 |
| 9 | 15.2 | 14.6 |
| 10 | 13.504 | 13.72 |
| Mean weight | 14.712 | 14.647 |
| **Bovine vitreous density = 1.005 ± 0.012 g/cm$^3$** | | |
| **Cow vitreous volume (cm$^3$) calculated from density & weight** | | |
| Cow | Left eye | Right eye |
| 1 | 14.98 | 14.67 |
| 2 | 16.12 | 15.60 |
| 3 | 14.89 | 15.05 |
| 4 | 14.41 | 14.30 |
| 5 | 14.41 | 14.81 |
| 6 | 13.90 | 14.38 |
| 7 | 15.18 | 15.23 |
| 8 | 13.93 | 13.51 |
| 9 | 15.12 | 14.53 |
| 10 | 13.44 | 13.65 |
| **Mean Volume** | **14.64** | **14.57** |
| **Mean Volume (all eyes)** | **14.61** | |
| **Standard deviation** | **±0.70** | |

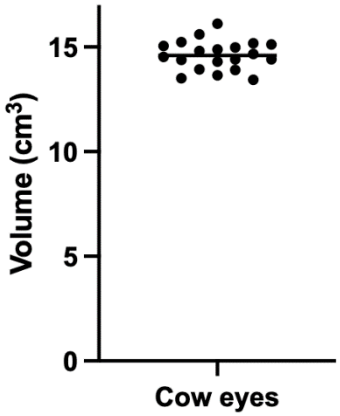
## Pairwise comparison of left vs right cow eye vitreal volume




| | Left | Right |
|---|---|---|
| Number of values | 10 | 10 |
| | | |
| Minimum | 13.44 | 13.51 |
| Maximum | 16.12 | 15.60 |
| Range | 2.681 | 2.089 |
| | | |
| Mean | 14.64 | 14.57 |
| Std. Deviation | 0.7807 | 0.6559 |
| Std. Error of Mean | 0.2469 | 0.2074 |

| | |
|---|---|
| Paired t test | |
| P value | 0.6070 |
| P value summary | ns |
| Significantly different (P < 0.05)? | No |
| One- or two-tailed P value? | Two-tailed |
| t, df | t=0.533, df=9 |
| Number of pairs | 10 |

## Vitreal volume of all 20 cow eyes




| | Cow eyes |
|---|---|
| Number of values | 20 |
| | |
| Minimum | 13.437 |
| Maximum | 16.117 |
| Range | 2.6806 |
| | |
| Mean | 14.606 |
| Std. Deviation | 0.70257 |
| Std. Error of Mean | 0.15710 |
| | |
| Lower 95% CI of mean | 14.277 |
| Upper 95% CI of mean | 14.935 |