# POSTER PRESENTATIONS

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# Assessing pain in models of Rheumatoid Arthritis

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## Introduction

A hallmark symptom of rheumatoid arthritis in humans is painful swollen joints. Pain can manifest before any inflammation is noticeable[1,2] as well as persist long after inflammation has resolved.[3]

In rodent models of arthritis, ankle or footpad width is a commonly used surrogate marker of pain (see Figure 1).

Measuring footpad width assumes that increased swelling is proportional to enhanced pain. A mild arthritis phenotype in which there is minimal swelling may therefore inaccurately reflect the extent of pain and discomfort.

**Aim: We aimed to determine how well pain correlated to footpad widths using the collagen antibody arthritis model.**

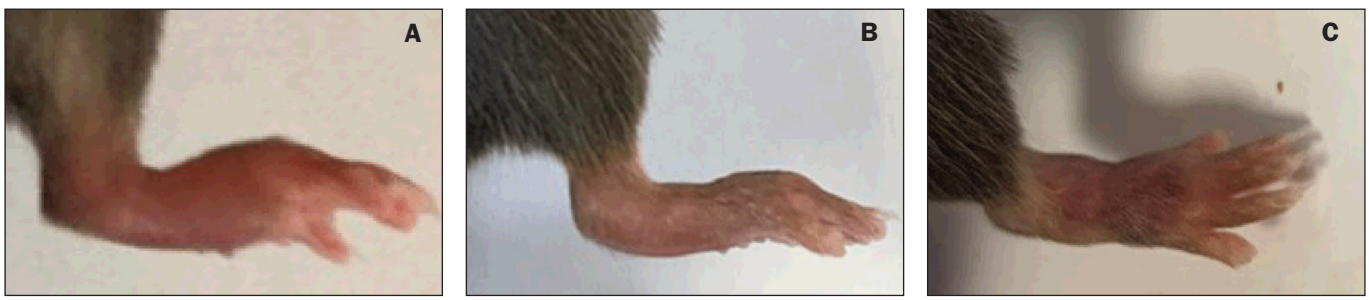



**Figure 1.** Footpad width as a surrogate measure of pain in arthritis models. Commonly used methods to assess pain are footpad width (**A**), ankle width (**B**) or footpad ankle length (**C**).

## Techniques to ease pain

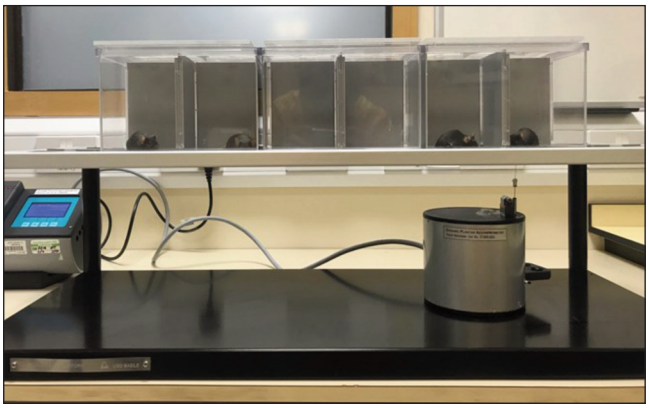



**Figure 2.** Equipment used to measure mechanical withdrawal thresholds (upper) and cold (<2°C) water hypersensitivity (lower).

## Collagen antibody induced arthritis (CAIA)

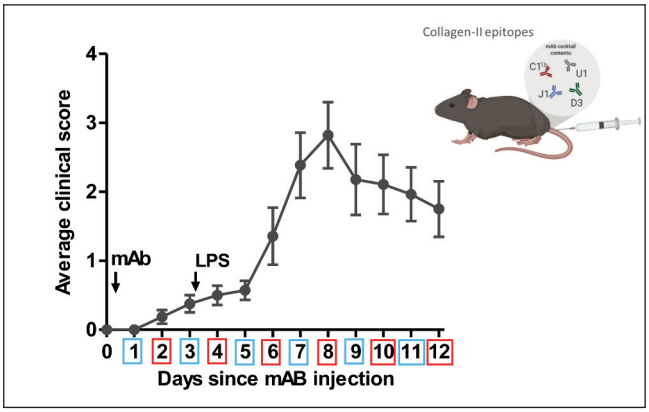



**Figure 3.** CAIA induction. A monoclonal antibody (mAb) is injected intravenously. Followed by an intraperitoneal injection of lipopolysaccharide (LPS) 3 days later.

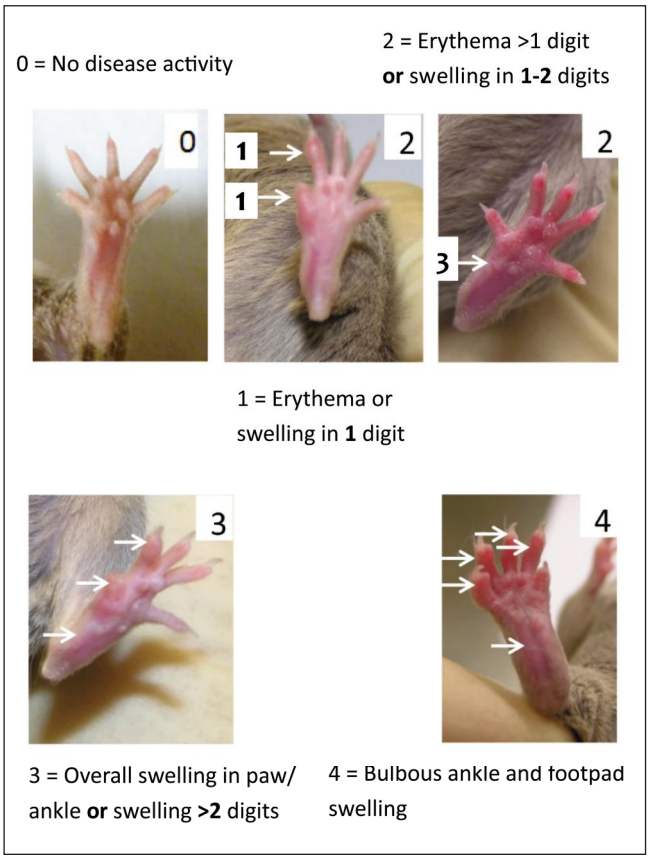
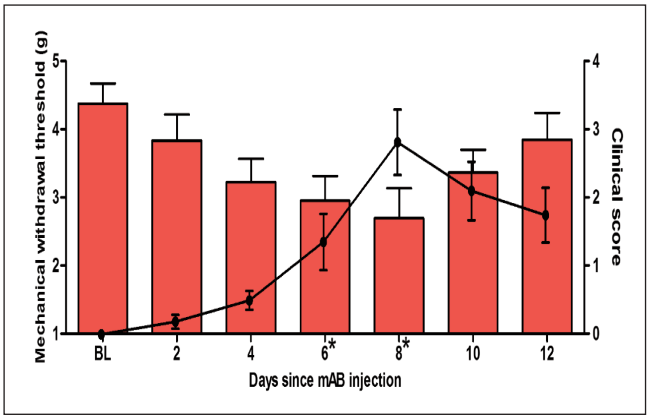
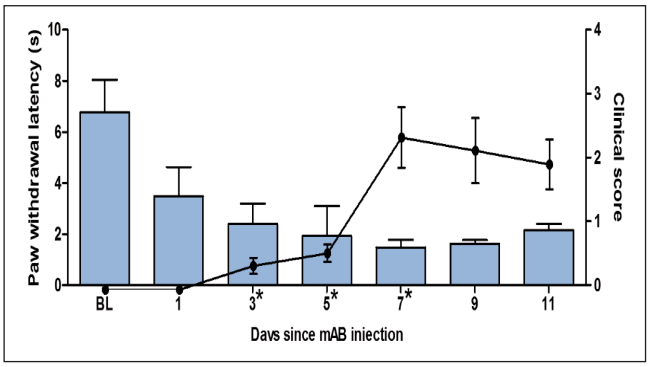
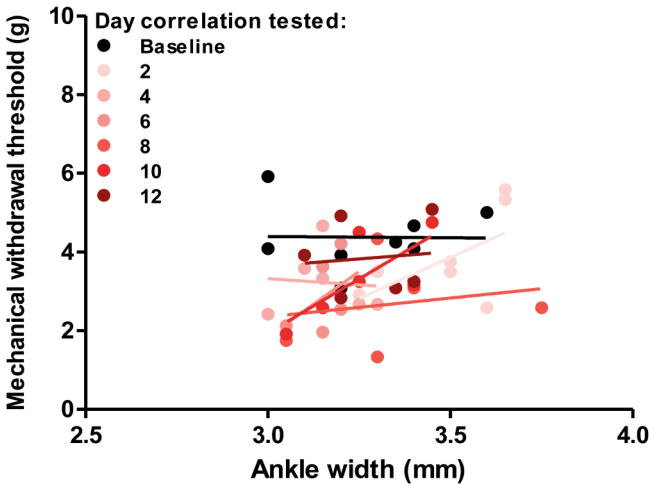



**Figure 4.** Clinical scoring. Example clinical scores observed in the paradigm (adapted from Coppard *et al.* (2019)[4] )

## Results

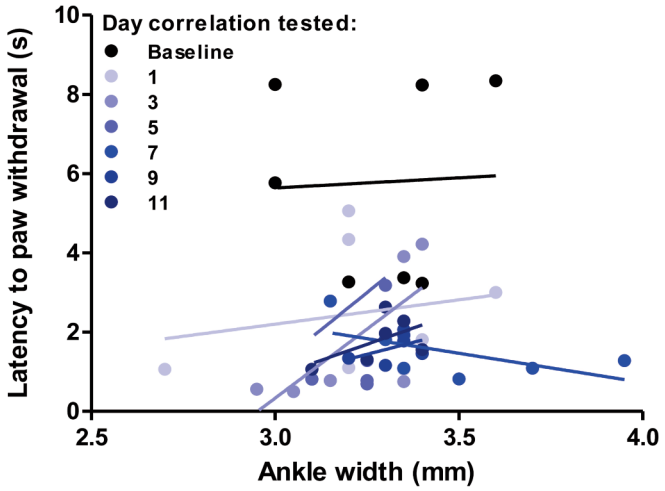





**Mechanical withdrawal threshold correlation to ankle width:**

| Day tested | Pearson's r value | P value | Significant? |
|---|---|---|---|
| Baseline | -0.015 (-0.71—0.70) | 0.97 | Not significant |
| 2 | 0.59 (-0.20—0.91) | 0.13 | Not significant |
| 4 | -0.08 (-0.84—0.78) | 0.88 | Not significant |
| 6 | 0.51 (-0.51—0.93) | 0.3 | Not significant |
| 8 | 0.20 (-0.73—0.87) | 0.7 | Not significant |
| 10 | 0.75 (-0.15—0.97) | 0.08 | Not significant |
| 12 | 0.10 (-0.77—0.84) | 0.85 | Not significant |




**Cold hypersensitivity correlation to ankle width:**

| Day tested | Pearson's r value | P value | Significant? |
|---|---|---|---|
| Baseline | 0.046 (-0.73—0.77) | 0.92 | Not significant |
| 1 | 0.20 (-0.65—0.83) | 0.66 | Not significant |
| 3 | -0.035 (-0.77—0.74) | 0.94 | Not significant |
| 5 | 0.86 (0.16—0.98) | 0.028 | Significant* |
| 7 | -0.6 (-0.95 - 0.41) | 0.21 | Not significant |
| 9 | 0.47 (-0.55—0.93) | 0.35 | Not significant |
| 11 | 0.56 (-0.46—0.94) | 0.25 | Not significant |

**Figure 5.** Pain in collagen antibody induced arthritis**. (A)** Time course of development of mechanical hyperalgesia. **(B and Bi)** Correlations between ankle width and mechanical withdrawal thresholds on each day of testing. **(C)** Time course of development of cold hypersensitivity. **(D and Di)** Correlations between ankle width and paw withdrawal latency from cold water on each day of testing.

In each case * indicates $P < 0.05$ from baseline.

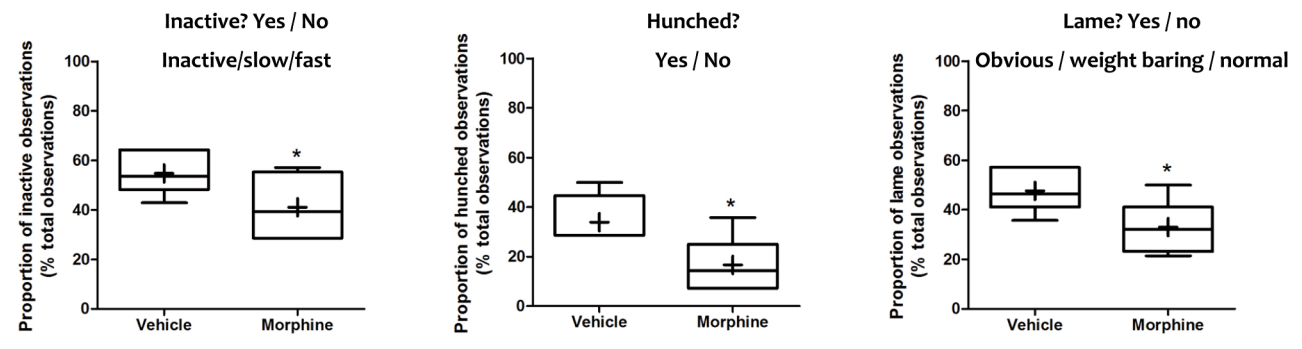



**Figure 6.** Affective measures of analgesia. Collagen antibody treated mice were scored as 'sad' as evident by their quiescence, hunched posture and lameness. Mice receiving morphine (3 mg/kg) displayed significantly fewer observations on all qualitative parameters. Additional observations that were scored included grooming status, ear position and piloerection (data not shown).

## Conclusions

Footpad widths poorly reflect pain experienced in arthritis models.

Observing the posture and activity of an animal might better indicate whether or not they are in pain.

Qualitative and semi-quantitative measures of affective pain can be useful in determining when an animal should receive analgesia.

Further qualitative refinements should be added to identify better humane endpoints.

## Acknowledgements

Studentship funding bodies:

BJA British Journal of Anaesthesia

RCOA Royal College of Anaesthetists

NIAA National Institute of Academic Anaesthesia

Thank you to all MRSU staff for their participation in affective scoring.

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# Refinements to health monitoring

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*Poster prepared for AST2020*

**Green health monitor**

- Weight: weekly (if cage is on a hand check already this will be done then)
- No other clinical signs
- Phenotype (related to strain)

**Amber health monitor**

- Weight: ≥ 10% loss
- Body condition: ≤2.5
- Sores/skin lesions: present but dry and no signs of scratching
- Papilloma present on face or genital region
- Anal prolapse: Up to 3mm protruding (Still pink, moist surface not bleeding)
- Respiration: rate and effort increased or decreased
- Tumours: visual or palpable or both
- Abnormal gait: wobbly or splayed or issues with movement. Intermittent hunching
- Pain: Signs of pain, but responds to analgesia
- Seizure: observed duration <30 secs
- General health: piloerection and underactive
- Diarrhoea (loose faeces)

**Red health monitor**

- Weight: Cachectic (≥20% weight loss) or loss of ≥15% weight due to diarrhoea (**HE**)
- Body condition: ≥2.0 (**HE**)
- Sores/skin lesions: exposure of dermal layer and/or covers an area bigger than a 5p piece
- Papillomas: prohibit normal bodily function, bleeding, or infected. Larger than pea sized (**HE**)
- Anal prolapse: >3mm protruding or Dark/ scabbed/bleeding
- Respiration: laboured, very increased or decreased
- Tumour: > 10mm diameter may vary depending on PPL. Multiple tumours palpable
- Gait: complete paralysis (one or more limb).Permanent hunched posture. Very jittery, inhibiting normal movement
- Seizure: third seizure observed (**HE**)

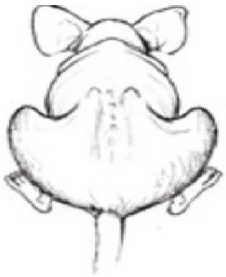
If a mouse shows any of the listed amber signs, then monitoring is increased to either once or twice weekly (green HC). Mice with 3 amber signs should be on red HC (daily check).

Mice which develop more than 3 ‘amber’ signs or any one red sign should be considered for humane endpoint (HE).

**Scoring the body condition of the mouse is also used for assessing the mouse as shown in the diagrams below. This is a non-invasive method of assessing mice such as tumour bearing models. Each assessment is matched to the description to give a body condition score.**

**BC1- Mouse is emaciated.**

- *Skeletal structure extremely prominent; little or no flesh cover.*
- *Vertebrae distinctly segmented*



**BC2 - Mouse is underconditioned.**

- *Segmentation of vertebral column evident.*
- *Dorsal pelvic bones are readily palpable.*

**BC3 - Mouse is well-conditioned.**

- *Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.*

**BC4 - Mouse is overconditioned.**

- *Spine is a continuous column.*
- *Vertebrae palpable with only firm pressure.*

**BC5 - Mouse is obese.**

- *Mouse is smooth and bulky.*
- *Bone structure disappears under flesh and subcutaneous fat.*

As mice age, they may develop general clinical signs that indicate dysfunction such as; gradual weight loss, stiffness/reduced mobility, sight deficits, unsteady/wobbly gaits and tumour growth.

# Biosecurity risks and the pre-implantation embryo: lessons from the mouse

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## Introduction

Today, the technique of embryo transfer is the most widely used method to rederive livestock that are microbiologically contaminated or to recover valuable animal lines archived by assisted reproduction techniques (ARTs).[1] In the mouse, standard process consisting in performing serial washings of preimplantation embryos in antibiotic supplemented media before embryo transfer (ET) has proved efficient in eliminating viral, bacterial, or parasitic infections.[2-6] Contamination may occur during embryos production and storage processes or could be mediated by gametes and embryos themselves.[7] Continuous training of the team in techniques and principles of disease controls, adequate facilities and equipment are thus mandatory to avoid accidental contamination. However, embryos may become contaminated *in vivo* at the oocyte stage prior to ovulation or following ovulation by exposure to microbial pathogens during transit through the oviduct and uterus and *in vitro* at insemination stage by spermatozoa when assisted fertilisation techniques are used.

Through analysis of scientific data relating to mouse species we discuss here the contamination risks linked to the oocyte/embryo and ways to prevent them.

## The embryo and contamination risks

The pre-implantation embryo is a proper entity in itself since it is present in the oviduct and uterus for only a very short time before collection without any vascular link to the recipient female at these stages. In addition, the embryo is surrounded by the zona pellucida (ZP) (Figure 1) which acts as a natural barrier against pathogens. The ZP is composed of a complex fibrous network of glycoprotein interspersed with numerous spores that are largest at the outer surface and decrease in size centripetally (Figure 2).

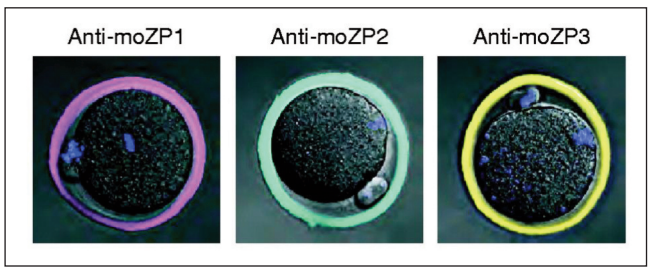


**Figure 1.** Mouse ovulated eggs stained with MABs specific to mouse ZP1, mouse ZP2 and ZP3 glycoproteins constituting the ZP (Confocalmicroscopy).[32]

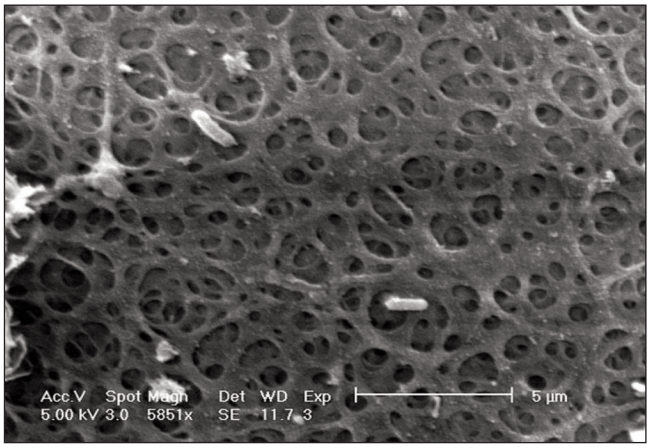


**Figure 2.** Scanning electron micrograph of the surface of the ZP of a hamster oocyte. Micropores formed by the complex structure of ZP1, ZP2 and ZP3 glycoproteins association are visible.
http://www.cellimagelibrary.org/images/12624

Because of their size, viruses are the most likely pathogens to be transmitted during ET since they may traverse the ZP or adhere firmly to the ZP by remaining lodged in its micropores thus rendering washing procedures less efficient in decreasing viral load. Very small viruses (27-30nm) such as autonomous parvovirus can traverse the ZP.[8-10] Mouse Minute Virus (MMV), (20 nm in size) can contaminate mouse embryos as confirmed by *in vivo* studies,[11,12] even when repeated embryos washing was performed.[11] In contrast, recipients of embryos arising from IVF with oocytes and MMV-exposed spermatozoa remained seronegative and were virus free, as well as their offspring.[11,13] In this last study prevalence of virus detected by real time PCR was lower in male than in

female reproductive organs and in 2C embryos derived from infected males than derived from infected females. For Mouse Hepatitis Virus (MHV,80-160 nm in size) no infection was observed after washing of embryos 10 times.[11] In endemically infected mice, Mouse Parvo Virus (MPV1) was detected in ovarian tissue, sperm cells, embryos and oocytes by Polymerase chain reaction (PCR), corroborating the transmission of viral particles.[14]

Presence of Lymphocytic choriomeningitis (LCMV) (60-300nm) in mouse oocytes and embryos has also been documented, providing evidence of transmission via the oocyte.[15] Sendaivirus (100–200nm size) was found in the murine ZP.[16,17] Finally, standard washing or trypsin treatment were not sufficient to remove Sendai virus adhering to the ZP of mouse blastocysts.

## The *in vitro* produced embryo

*in vitro* produced embryos may be exposed to specific contamination risks.

## Infected sperm

Presence of prevalent pathogens (Helicobacter typhlonius, MHV, MPV and retroviruses) in gonads and gametes has been demonstrated.[14,18-20] Efficient methods to remove pathogens from mouse sperm are not yet available. However, the risk of sperm mediated contamination in IVF using cumulus enclosed oocytes from non-infected females seems negligible.[13,18,19] Cumulus cells seem to exert a protective effect against pathogens. Embryos arising from IVF of cumulus enclosed oocytes with MMV-exposed sperm did not lead to seropositive recipients and pups, in contrast to IVF of cumulus denuded oocytes.[22] When cumulus cells are removed from the ZP, micropores to approximately 140-1000nm,[23] arise thus allowing entrapment of a higher quantity of the virus from the fertilization media. Cumulus cells may also absorb some of the viruses thus contributing to decrease of the viral load.[14]

In addition, standard washing procedures were effective in avoiding MHV transmission when sperm and oocytes were incubated with high levels of virus.[23] Additional studies are needed to determine whether other prevalent viruses can be transmitted by sperm and IVF.

**Invasive ARTs** such as laser assisted IVF and intracytoplasmic sperm injection (ICSI) use cumulus-free oocytes for facilitating the micromanipulation and lead to ZP rupture. Thus, the oocyte protection against potential contamination is lower. However, the number of sperm cells in contact of the oocyte is also far less than in conventional IVF. On the other hand, for some viruses such as MHV, contamination could be avoided by standard repetitive washing procedure when zona pellucida was partially disrupted by laser microdissection.[24] More research is needed to assess the risks with other pathogens since very few data in mouse are available in this field.

**ES cell injection** following transfection with desired gene constructs coupled with ET is a standard method for producing transgenic mice. Mouse ES cells (mESC) may harbour infectious pathogens. The most prevalent mESC contaminating agents are mycoplasma and viruses such as lactic dehydrogenase elevating virus (LDEV), polyomavirus, LCMV and parvo viruses. Mycoplasma may affect various mESC and parameters, germ line transmission, and postnatal development of resulting chimeras.[25] Few data are available regarding pup contamination through injection of contaminated EScells. Pup contamination was observed after injection of mESC infected by MMV but not by MHV, MHV being cytolytic form ESCs.[26] Infection risk can be significantly reduced by generating ES cells from mice free of relevant pathogens and by testing the ES cells before injection.

**ZP structural alteration** may arise from *in vitro* culture of mammalian preimplantation including the mouse.[27,28] In pigs and bovines some pathogens seem to adhere more readily to IVP embryos.[23,29] Such interactions might differ from one pathogen to another and from one species to another. No data has yet been published in the mouse supporting this effect.

## Embryos/gametes storage and transportation

Liquid nitrogen during its storage and distribution may become an effective media for the cryopreservation of fungal spores, yeasts, bacteria and viruses and may lead to contamination of embryo and gametes stocks.[30] Sealed samples of embryos stored in contaminated liquid nitrogen tanks tested negative for the presence of bacteria or viruses.[31] Similarly, the vapour phase of liquid nitrogen is a safe means for the short-term storage and transportation of embryos in dry shipper dewars.[31] For review on the risks of contaminating germplasm during cryopreservation and cryobanking see Yauger, *et al* 2011.[32] It is important, therefore, to prevent direct contact of germplasm and reproductive tissues with LN during cryopreservation and their storage. This includes the usage of hermetically sealed high-quality straws or cryotubes. A periodic disinfection of cryo-Dewars should be considered as an additional precaution to diminish the potential for inadvertent cross-contamination. Separation of LN Dewars to quarantine embryos derived from infected donors of valuable genotypes or from unknown health status is also advisable.

## Conclusion

Rederivation procedure using embryo transfer is an efficient technique for pathogens removal in livestock

animals and the mouse. Maximum efficiency is reached when special care is taken using zona intact preimplantation embryos and sufficient washing of oocytes and embryos (at least 10 wash steps, dilution factor 1:100 per wash step). Nevertheless, viral infection may occur since viral particle size may allow them to traverse or adhere firmly to the ZP thus rendering washing procedures less efficient. The risk seems restricted to very small prevalent viruses such as MPV and MMV. Viral contamination risk seems higher when using biological material (oocytes/embryos) originating from contaminated mouse females than from males. By consequence, when using oocytes/embryos from contaminated females a quarantine should allow dramatic reduction of viral load in reproductive organs and safe rederivation procedures through ART. Contamination through sperm in IVF procedure appears to be negligible so this approach should be favoured when possible. The use of 2-cell stage embryos is also recommended for ET because viral DNA could not be detected by PCRin2-cell embryos derived from MMV *in vivo* infected mice after sufficient washings.

More and more studies are using PCR-based techniques for detection of pathogens. Detection of viral DNA by a very sensitive method does not necessarily imply the presence of sufficiently infectious virions at embryo level.

Nonetheless, because seroconversion to virus (ex parvovirus) depends on several factors, including mouse strain, stringent hygienic measures should be implemented, including recipients being kept in IVCs or isolators with screening before release of the rederived pups.

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# Environmental enrichment for a small colony of rats

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## Introduction

The Biological Resources Unit at the Cancer Research UK Cambridge Institute predominantly houses mice with a small number of rats used for brain cancer studies, due to limited space and resource for equipment we came up with some easy and cost-effective ways to improve enrichment for the rat colony. Since implementing our protocol for forage feeding and out of cage enrichment, we have found the rats easier to handle and more confident. This has proven invaluable with regards to limiting stress whilst they are 'visiting' our imaging facility.

**Figure 1.** Approximately twenty rats are housed in IVCs in the same holding room as mice.

**Figure 2.** Forage Mix (LBS-Biotechnology).

A small amount of forage mix is buried in the bedding material of the rat's home cages during cage cleaning and daily checks, as they spend time foraging until they have found and eaten all of the food. We also hand feed the rats to encourage human interaction and this helps us monitor health effectively as they have to reach up to the cage door to take it.

We provide our rats with irradiated LBS forage mix (LBS Biotechnology), which contains ingredients known to be appetising to rodents such as sunflower seeds, banana, maize flakes and locust beans (Figure 2).

Rats are provided with supervised out of cage enrichment in a larger cage that is small enough to fit inside a downdraft cabinet.

Introducing this new environment regularly provides a positive association with leaving the home cage.

**Figure 3.** Rats in 'out of cage' environment.

All enrichment must be easily cleaned or disposable to avoid cross contamination. We use disposable fun tunnels, also Perspex Jolley balls and screens that can be easily disinfected with Vaporised Hydrogen Peroxide (VHP) or autoclaved. Rats also enjoy playing with empty glove boxes, a 'green' and cost-effective way to provide extra enrichment for your animals.

# Oestrus checking – increasing productivity and embracing the 3R's

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## Overview

Much like the menstrual cycle in humans, mice also have a cycle where the eggs ripen, ovulation takes place, the females come into heat and are receptive to mate with the male, this occurs over the space of 4-5 days and is called the oestrous cycle. The oestrous cycle in mice has four stages: pro-oestrus, oestrus, met-oestrus and di-oestrus. The appearance of the epithelium of the external genitalia is used to identify the stage of the oestrous cycle in a female mouse. Females are selected in the afternoon when they are in pro-oestrus and mated overnight. The females are then plug checked in the morning and if a plug is found this confirms mating has taken place.

## Previous results and aim of the study

An original trial performed at our legacy site in 2011 showed the rate of successful matings after implementing this method of oestrus checking increased from 13% to 54%, when compared with randomly selecting females for mating. This method was then used in successfully re-deriving all of our mouse strains in preparation for moving to The Francis Crick Institute in 2017 (The Crick).

Initially at The Francis Crick, timed matings were set up using dirty bedding to induce the Whitten Effect* and had a low success rate. It was decided to perform a new trial of oestrus checking with the aim of increasing the number of confirmed matings and improving consistency in fulfilling timed mating orders for the Genetic Modification Service (GeMS) and other users. If successful, it would allow us to reduce the number of mice used and the need to repeat unsuccessful matings in accordance with the 3Rs whilst also allowing us to decrease the waiting times for projects of work through the GeMS team.

* **Whitten Effect** – the stimulation by male pheromones, of synchronous oestrus in a female population, in this case the addition of dirty bedding containing male mouse pheromones to the female mouse cage.

This trial in early 2018 showed an average successful timed mating rate of 81% in CD-1 females and it was decided to adopt this method for all A2 timed mating orders going forward. We have seen a large increase in success rates with other mouse strains by using this method as well.

## What is oestrus checking?

Oestrus checking, as we call it, is a visual check of the female's genitalia to assess her readiness to mate.

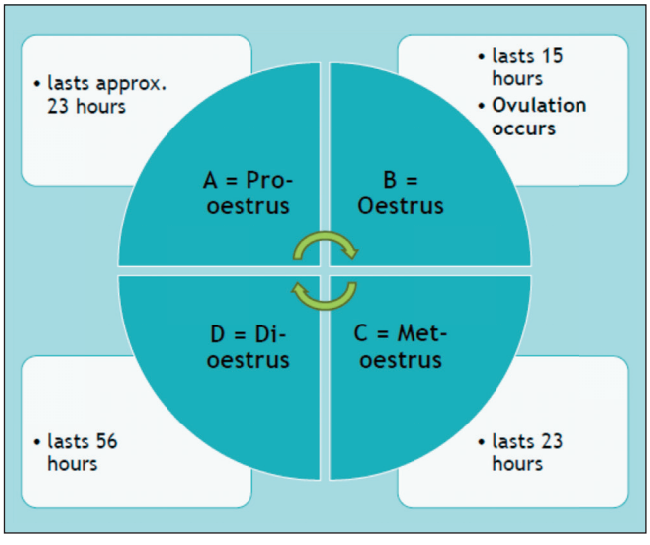
It is done by holding each female by the base of the tail in your left hand and using the thumb of your right hand, gently touch the stomach of the mouse to open the vagina to assess which stage of the cycle she is in.

Females that are at the correct stage are then mated with the males for one night only.

## Oestrous cycle

There are 4 stages of the oestrous cycle in mice and altogether the cycle lasts 4-5 days.

For mating purposes, we are looking for the pro-oestrus stage.



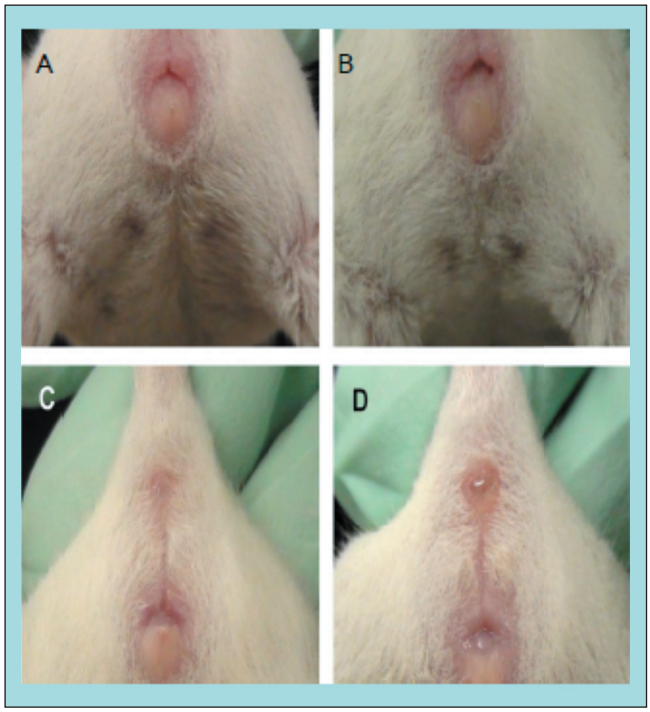



**Figure 1**: Visual appearance at each stage of the oestrous cycle.

## Signs to look for in pro-oestrus

- very swollen puffy tissue
- bright pink colour
- striations or lines
- moist tissue

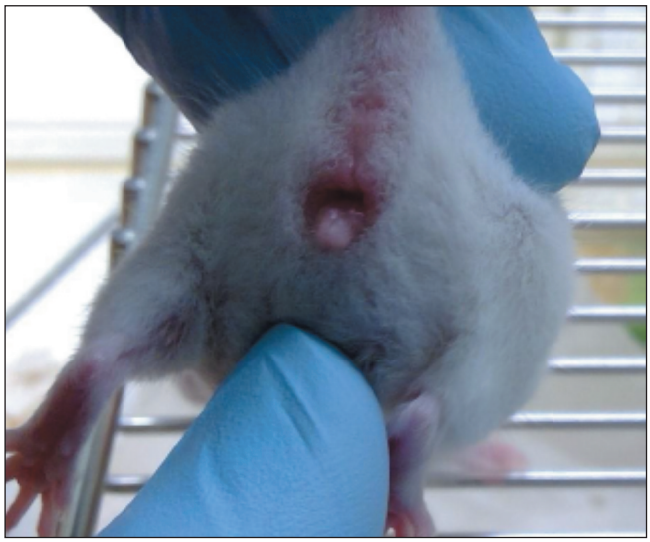


**Figure 2.** Female in pro-oestrus

Pairing takes place in the afternoon; this is because with a normal 12/12 light cycle the female and male are put together at the end of the light period. The mating is expected to take place early in the dark period, plug checking is performed first thing in the morning to confirm that mating has taken place.

## Plug checking

When mating occurs, the male ejaculate forms a solid mass inside the female's vagina. We call this a plug.

We check for this plug to confirm that mating has taken place. We use a thin metal stick to feel for the plug but often you can see it without using the stick.

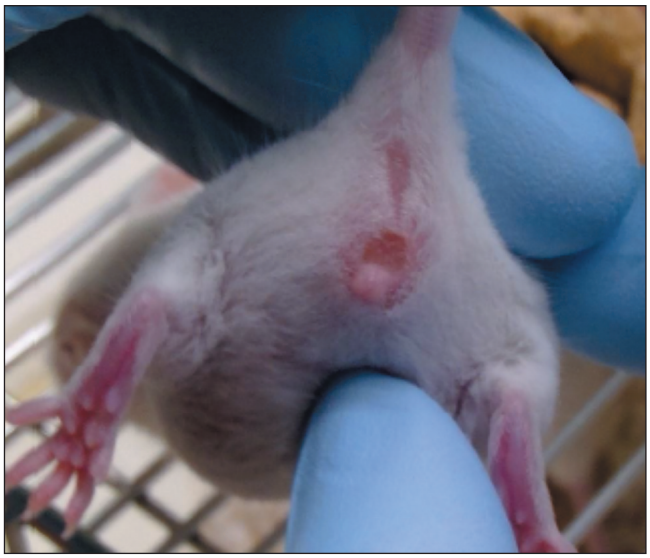


**Figure 3:** Plugged female.

## Results

*Random selection of females*

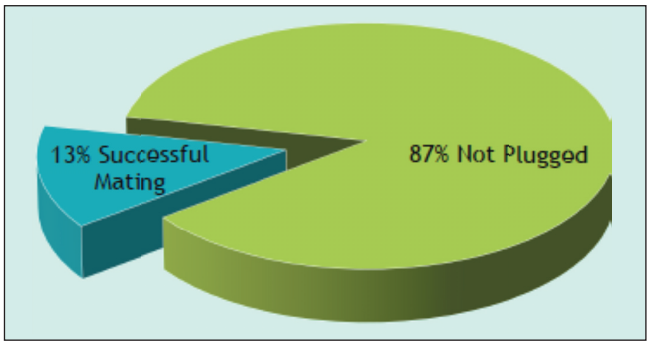



**Figure 4.** Chart showing the percentage of successful matings without using the Whitten effect or oestrus checking.

*Use of Whitten Effect*

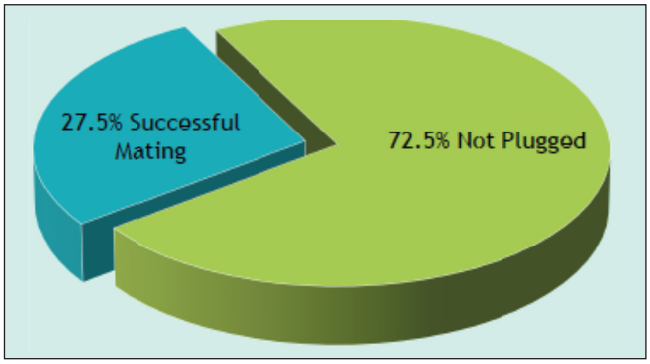



**Figure 5.** Chart showing the percentage of successful matings using the Whitten Effect.

*Use of Oestrus checking*

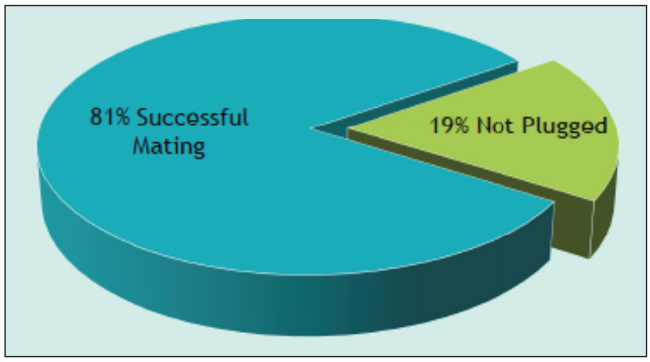



**Figure 6.** Chart showing the percentage of successful matings using oestrus checking.

## What are the benefits of oestrus checking?

- Oestrus checking allows us to only select females that are ready to mate thereby reducing the number of mice being used for mating.
- This means that we can be very efficient, ensuring we have plugged females on the requested days, filling orders for a variety of users and strains.
- It also allows us to tailor the numbers being mated to fulfil our orders without over breeding.
- GeMS require large numbers of both donor and recipient females daily, at both E0.5* and E2.5*. These need to be supplied as ordered so that GeMS can plan and execute their work.
- Oestrus checking allows us to supply these consistently and meet the ever-increasing demand.

## How oestrus checking impacts GeMS work

- Consistently obtaining the plug numbers required allows the GeMS' team to plan their work more efficiently.
- Birth rates are improved if embryo thaws and implantation takes place quickly.
- Embryo numbers are predictable allowing GeMS to have the correct number of injectable blastocysts ready.
- Predictable yields allow GeMS to schedule when a cell line is likely to be injected, minimise culture times and give accurate timelines to users.
- It is essential to allow GeMS to deliver a good service and reduce waiting times.

* E0.5 and E2.5 refer to days post-fertilisation

## Conclusion

Selecting females for mating by visually checking for oestrus vastly increases successful plugging rates in CD-1 females. Our results show an increase from 13% to 81% by introducing this method into our daily routines.

- This allows us to greatly reduce the number of matings needed to produce the desired number of plugs in accordance with the 3R's and to consistently hit plugging targets to fulfil orders.
- Choosing only females that are ready to mate also reduces the stress levels of having females not suitable for mating being introduced into male cages. This is a good refinement to the process as it minimises the risk of injuries caused by aggression from the males. Anecdotally it results in a higher confirmed pregnancy rate from plugged females.
- Providing a stable and dependable service to the GeMS' team and other users allowing them to plan work based on predictable yields has allowed them to give accurate timelines to users for their projects, reduce waiting times and clear the backlog that had built up before oestrus checking was introduced.
- Oestrus checking all of our plugging orders is very time consuming and initially caused scheduling issues with having trained people available during weekends. However, it is relatively easy to train staff to use this method and once they have picked up the skill and are proficient it allows us to provide a consistent high plugging success rate even at weekends and holidays.
- Despite the time taken and increase in workload that oestrus checking brings, it has proven to be invaluable in allowing us to consistently fulfil our plugging orders and provide this service to the GeMS' team and our other users.

## Acknowledgements

Thanks to Ian Rosewell and Sophie Wood for their input on how oestrus checking impacts GeMS work.
Thanks also to Helen Bailey for help with poster design and content and Peter Miller for technical help with PowerPoint to produce the diagrams.

## References

**Figure 1:** taken from https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0035538

# Using habituation to reduce stress for rats being transported short distances

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## Introduction

Transport in rats has been shown to be a big stressor, this results in some major changes in their physiology.[1]

More recent research has found that the immune system can also be affected in addition to increased corticosterone level which affects heart rate, blood pressure and immune function as well as behaviour [2,3]

Animals need to return to their normal baseline physiological parameters of homeostasis before they are used for experimental procedures.[2] The physical stress of movement can cause muscle damage during travel. Moreover, their behaviour is affected in that their exploration and grooming behaviour is reduced.[3]

Research shows that even husbandry procedures cause acute amounts of stress to laboratory animals. It has also been shown that moving rats to another room for a procedure is more stressful than if they are in their holding room having the same procedure.[4]

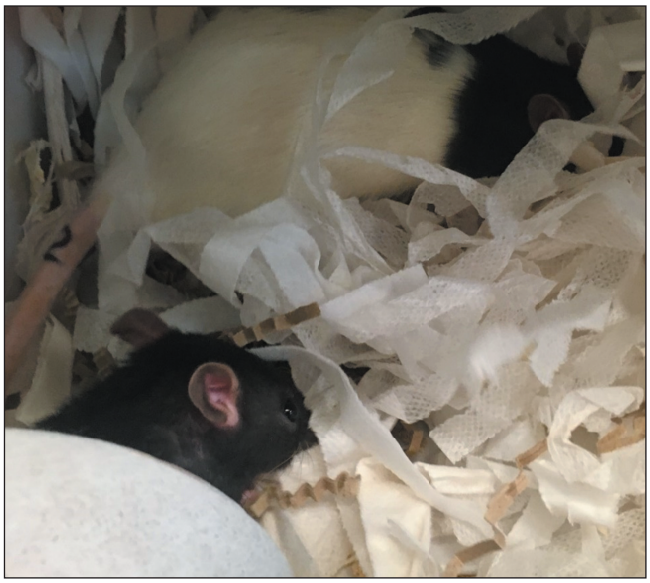
Swallow., et al. 2005[5] states that potential sources of stress for animals that are undergoing transport can also include:-

- handling.
- separation from cage-mates
- movement – vibrations during transport causing physical stress with adjusting balance.

## Method

According to Arts *et al.,* 2012, stressed animals can affect the reliability and validity of research result.[2] To reduce the stress experienced by the rats during transport, I felt that the rats needed to be familiar with the transport box. I was informed by a researcher that her Lister Hooded rats were hard to work with after travelling in a box, even for the short distance from the facility to the laboratory on the floor below.

**Figure 3.** Rats in transport box.



**Figures 1 & 2.** Rat habituating with transport box in the ball pool.

To familiarise the rats to the transport box, I built an empty one and placed it into the ball pool allowing the rats to get some exercise and play. I placed it in the ball pool with the lid open and hid some treats amongst the bedding.

The rats were keen to investigate and each time the rats went close to the box or touched it I gave them a small treat.

After several days of repeating the procedure the rats were climbing into the box and jumping in and out of it. Once the rats showed no signs of fear of the box in the ball pool I then moved it into their home cage and repeated the procedure. After doing this for several more days the rats became familiar with the box and were no longer fearful of it and used it as part of their enrichment within their cage.

## Results

When the rats were needed and the researcher took the rats to her laboratory to carry out procedures, she found that the rats were a lot calmer and easier to handle after being trained with the transport box.

## Conclusion

In order to find out if the stress of being transported has been reduced by the acclimatisation to the transport box I would need to do further investigations and collect data. This would involve a scoring system of the behaviour of the rat and their interaction with the box at each stage of the training process and the experimental procedure once they have been transported.

On initial investigation it seems that if the rats are familiar with the transport box this can reduce the stress they experience when being transported.

Further investigation is needed to find out if this works in other strains of rats and for longer periods than that of just being transported from one floor to another in the same building.

**Figure 4.** Rats searching for hidden treats.

**Figure 5.** Transport box placed in home cage.

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# Shining a light on rearing pigmentless Zebrafish

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## Introduction

Zebrafish (*Danio rerio*) strains with mutated pigment cells allow clear *in vivo* imaging to be carried out. In 2016, Crystal mutants, which lack most pigment cells in both their body and eyes, were first produced at King's College London.[1]

However, evidence shows strains with mutated pigment cells exhibit reduced survival during the early rearing stages in comparison to wildtype strains.[2,3] The Crystal strain, in particular, has poor survival in the nursery compared to wildtypes and anecdotal evidence suggests lower spawning success compared to other strains.

Light has been shown to influence the survival of wildtype zebrafish larvae,[4] however research of its effect on other zebrafish strains is limited.[3] Light is also well known to influence spawning behaviour in zebrafish, as their reproductive cycle is photoperiod-dependant, with most spawning at dawn.[5]

This experiment aimed to look at the impact of light intensity as to **refine** methods of rearing pigmentless Zebrafish and to **reduce** the number of fish needed to maintain important genetic lines, such as the Crystal line.

## Materials and Methods

Three strains of Zebrafish, varying in their degree of pigmentation, were selected (Wildtype AB, Albinob4 and Crystal Mutant (nacrew2/w2;albb4/b4;roya9/a9)).

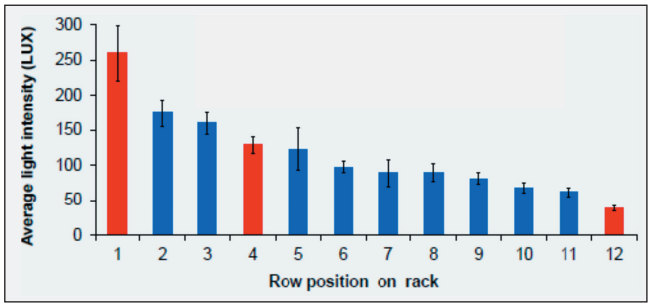


**Figure 1.** Average light intensity (LUX) of tanks across two racks. High, medium and low light intensity used for the purpose of this study are shown in red.

Twelve rows, on the same recirculating system, were filled with empty tanks. A lux meter was used to calculate the average light intensity (LUX) of each row from inside the tanks. Three rows were selected to represent high (259 LUX), medium (128 LUX) and low (38 LUX) light intensity levels (Figure 1).

Three tanks of each Zebrafish strain were then assigned to each of the three rows. At 5dpf, fry were transferred into 3.5 litre tanks (n=25 per tank), containing 1 litre of water. All fish were fed according to methods previously described by Mantzorou *et al.* (2017).[6]

Upon reaching sexual maturity (between 58-84dpf) the survival rate of each tank was calculated. All tanks were then reduced to 17 fish per tank. Remaining fish were then paired to spawn, a total of three times, to measure spawning success. Eggs from each pair were collected in separate petri-dishes and the fertile eggs produced by each pair were counted.

**Figure 2.** Wildtype AB (left), Albino (centre) and Crystal Mutant (right) Zebrafish strains.

## Results

Of the groups tested, zebrafish strain was found to significantly affect the survival rate of zebrafish during the nursery period ($p<0.01$) and the survival rate of each strain was found to be significantly different to each other ($p<0.01$). In contrast, light level had no significant effect on survival rates in any of the zebrafish strains studied (Figure 3).

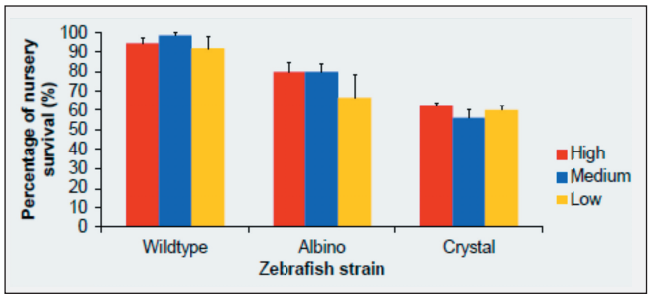


**Figure 3.** Average survival of three Zebrafish strains during the nursery period when exposed to three lux levels. Error bars show standard error.

Spawning success was also significantly affected by the strain of zebrafish ($p<0.05$), with a significant difference found between wildtype and crystal spawning success ($p<0.01$). In comparison, light level had no significant effect on spawning success in any of the zebrafish strains studied (Figure 4).

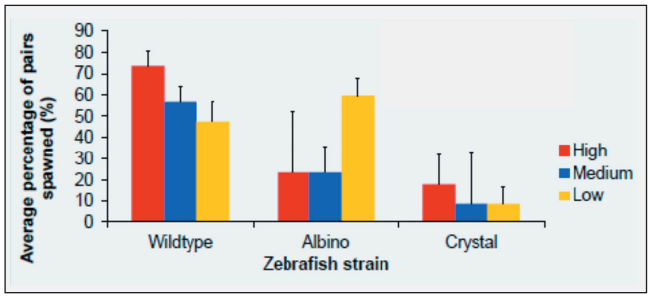


**Figure 4.** Average spawning success of three Zebrafish strains raised at three lux levels. Error bars show standard error.

There was no significant effect of either zebrafish strain or light level on the fertility of any of the zebrafish studied (Figure 5).

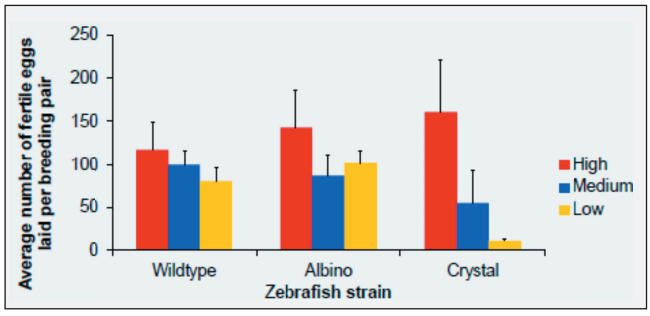


**Figure 5.** Average number of fertile eggs laid by three Zebrafish strains raised at three different lux levels. Error bars show stand error.

## Discussion

The results demonstrate that **crystal mutants exhibit significantly lower survival rates** (Figure 3) in comparison to albino and normal pigmented wildtype strains. The current study suggests lower levels of pigmentation to be associated with lower survival in zebrafish, which is in line with previous studies. [2,3]

It can also be seen that the **average number of spawning crystal pairs was lower than for either wildtype or albino strains** (Figure 4). Wildtype and crystal strains show a trend for increased spawning success at the highest LUX level, whilst albino zebrafish spawned best when housed at the lowest LUX level.

The study found **no significant difference in the average number of fertile eggs laid per strain**, which is in agreement with findings of Antinucciand Hindges (2016).[1] All strains showed a tendency for increased fertility when housed at the highest LUX level (Figure 5). To conclude:

- Survival was significantly affected by zebrafish strain, but not light intensity.
- Fertility was not significantly affected by strain or light intensity.
- Spawning success was not significantly affected by light intensity.
- Crystal mutants have significantly lower spawning success compared to wildtypes.

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# The Jacket with pulling power – a novel approach to early stage evaluation of magnetic nanoparticles

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## Introduction

As a cancer research group, one of our aims is to develop more efficient methods of compound delivery. One area we are investigating is that of the delivery of **theranostic nanoparticles**, which combine both therapeutic and diagnostic agents that directly target a tumour, thus reducing potential side effects from systemic therapies.

One delivery method is to magnetise the nanoparticles during formulation and, after injection, they are exposed to a magnetic field located close to the tumour (Figure 1).

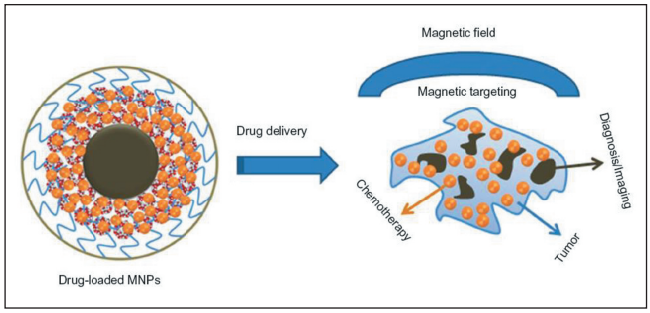


**Figure 1.** Principles of theranostic particle delivery.

In order to study the targeting efficiency as well as the theranostic properties of these magnetised nanoparticles, they need to be tested *in vivo.*

Researchers have used a variety of methods to expose the particles to a magnetic source, including –

i. MRI-expensive, therefore impractical for early stage testing, requires lengthy anaesthesia and specialist equipment.
ii. Sticking a magnet to the animal's skin with tape or superglue-can damage the skin and is difficult to remove.
iii. Surgically implanting subcutaneously-requires full aseptic surgery and remains in place until termination.

However, it is very difficult to reproduce reported results as methodology details are scant and often rely on cartoons rather than photographs (Figure 2).

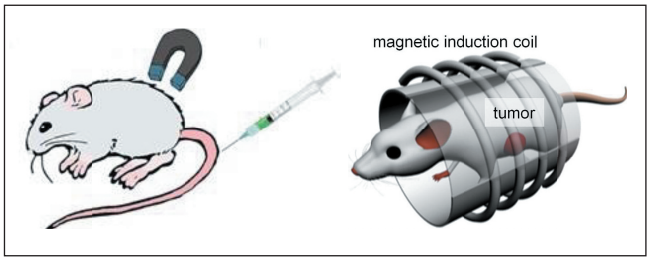


**Figure 2.** Representations of *in vivo* testing of theranostic particles.

## Materials and methods

We wanted to develop an early stage testing system which would allow us to fine-tune the formulation of our nanoparticles prior to full translationally-relevant testing and which would demonstrate the efficiency of the targeting without the need for invasive, damaging or uncomfortable procedures.

To this end, we decided to develop a novel, inexpensive and non-harmful way to present the magnet to the tumour and took our inspiration from the already widely-available infusion jacket.

Working with the manufacturer, we designed a Spandex jacket (Figures 3 and 5) with a pocket to house the magnet (Figure 4) and proceeded to test our nanoparticles.

By developing this, we hoped to deliver the particles intravenously and then direct them towards an established subcutaneous tumour using the magnet.

After injecting the magnetic nanoparticles intravenously via the tail vein, the mouse was lightly anaesthetised with an injectable anaesthetic, the jacket was fitted and remained in place for one hour (Figure 6).

**Figure 3.** Skin side of jacket.

**Figure 4.** Magnet.

**Figure 5.** Outside of jacket.

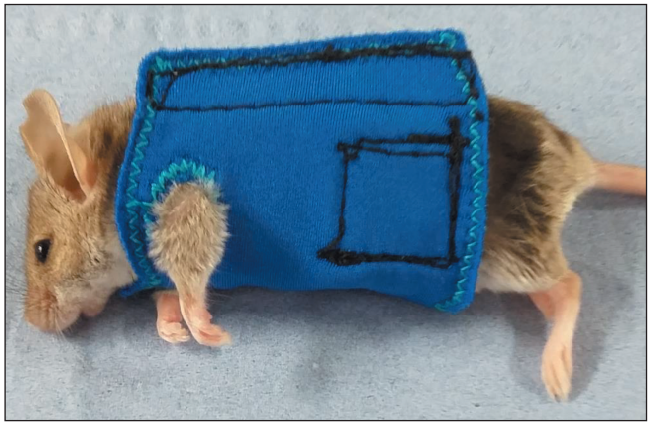

**Figure 6.** Mouse wearing jacket.

After this time, the jacket was removed and the animal was imaged for fluorescence in the IVIS Spectrum (Figure 7) under the same anaesthetic event.

**Figure 7.** IVIS Spectrum imaging system.

## Results

I hr post injection fluorescent images

**Figure 8.** With magnet.

**Figure 9.** Without magnet.

It can be seen from the images that the Cy5 fluorophore conjugated magnetic nanoparticles have accumulated in the region of the tumour in the presence of the magnet (Figure 8) but not in its absence (Figure 9).

However, *ex vivo* analysis showed that the particles had not actually penetrated the tumour, which we believe is due to the encapsulated nature of the subcutaneous tumour.

## Conclusions

Preliminary data is very promising in that it demonstrates the ability of the magnet to attract the magnetic nanoparticles when housed in the jacket. Moreover this pilot study has also identified a number of areas for improvement, particularly in facilitating tumour uptake of the nanoparticles. To this end, we intend to further test the system against other tumour models, including an orthotopic intraperitoneal model.

However, we believe that with some further refinements, this system could be used to quickly and easily test different nanoparticle formulations, giving us the opportunity to improve and reformulate them prior to taking them forward into more translationally relevant studies, while ensuring high welfare standards for the mice throughout the procedure.

## Acknowledgements

Thanks to LOMIR for their assistance in developing the jacket and to Marian Meakin and Alison Mackie for their technical assistance.