

“SHE’S JUST A FRIEND!”

- vas stud males and companions.

by Agenda Cambridge staff

At the University of Cambridge Facility, genetically altered mice for PetMedix Ltd are being generated by microinjection. Embryonic stems cells carrying the desired genetic alteration are injected into 3.5dpc pre-implantation mouse embryos, before being transferred back into pseudopregnant recipient females who will carry the embryos to term. To generate the embryos and recipients required we have a number of dedicated processes utilising a non-vasectomised stud bank and a vasectomised stud bank which consist of singly housed male mice.

We have pools of embryo donor females and pools of embryo recipient females co-housed in cages of up to 6. Between once and twice a week, depending on schedules, we can oestrus select the donor and recipient females through visual inspections of the vulva which are then mated 1:1 with the studs overnight. The embryo donor females are mated with the non-vasectomised studs and the embryo recipient females are mated with the vasectomised studs to render them pseudopregnant. The embryos are harvested 3.5dpc and microinjection takes place. The male studs are singly housed for 5-6 days per week and only have a companion for between 18-44 hours per week.

Reduction;

The stud banks are managed to ensure the minimum number of studs required are housed at any one time. Therefore, each male will have a female to mate with each week and will not go for extended periods without being used as a stud.

Refinement;

Mice are social animals, so being singly housed is a compromise to their welfare. Once males reach sexual maturity they are separated from their cage mates to enter the stud bank. Due to aggression the male mice cannot be co-housed back with cage mates in-between matings.

We are unable to house females with the non-vasectomised studs as this would produce unwanted litters. Stuart Newman (PetMedix) had seen the 2019 ISTT 3R Awardee work on co-housing companions with vasectomised stud males so suggested we trial implementing this in our facility as a refinement.

Concerns;

- Would the vasectomised stud males stop mating the recipient female when housed with a companion female? This would compromise the science.
- Would the success rate drop? Would we require more surgically vasectomised males in the stud bank to achieve the number of plugged females?
- Would identifying the companion and recipient be labour intensive?
- Would additional mice need to be bred to provide companions?

The Project;

The recipient mice are a B6CBAF1 which have an agouti coat colours. We decided to select albino companion females, this would ensure no further identification methods are necessary to differentiate between the companion and recipient female. Embryo donor females have an albino coat colour and at 14 weeks of age they are retired from the pool. These females made excellent companions so no additional breeding has been required to produce companions.

We started by housing a female companion with a the vasectomised stud in 10 cages and compared the plug success rate to that of stud without a companion.



Embryo recipient female mouse (agouti) has been oestrus selected and placed in vasectomised stud males cage. Female companion (albino) in tunnel.



Vasectomised stud male, companion and embryo recipient female housed together for the night

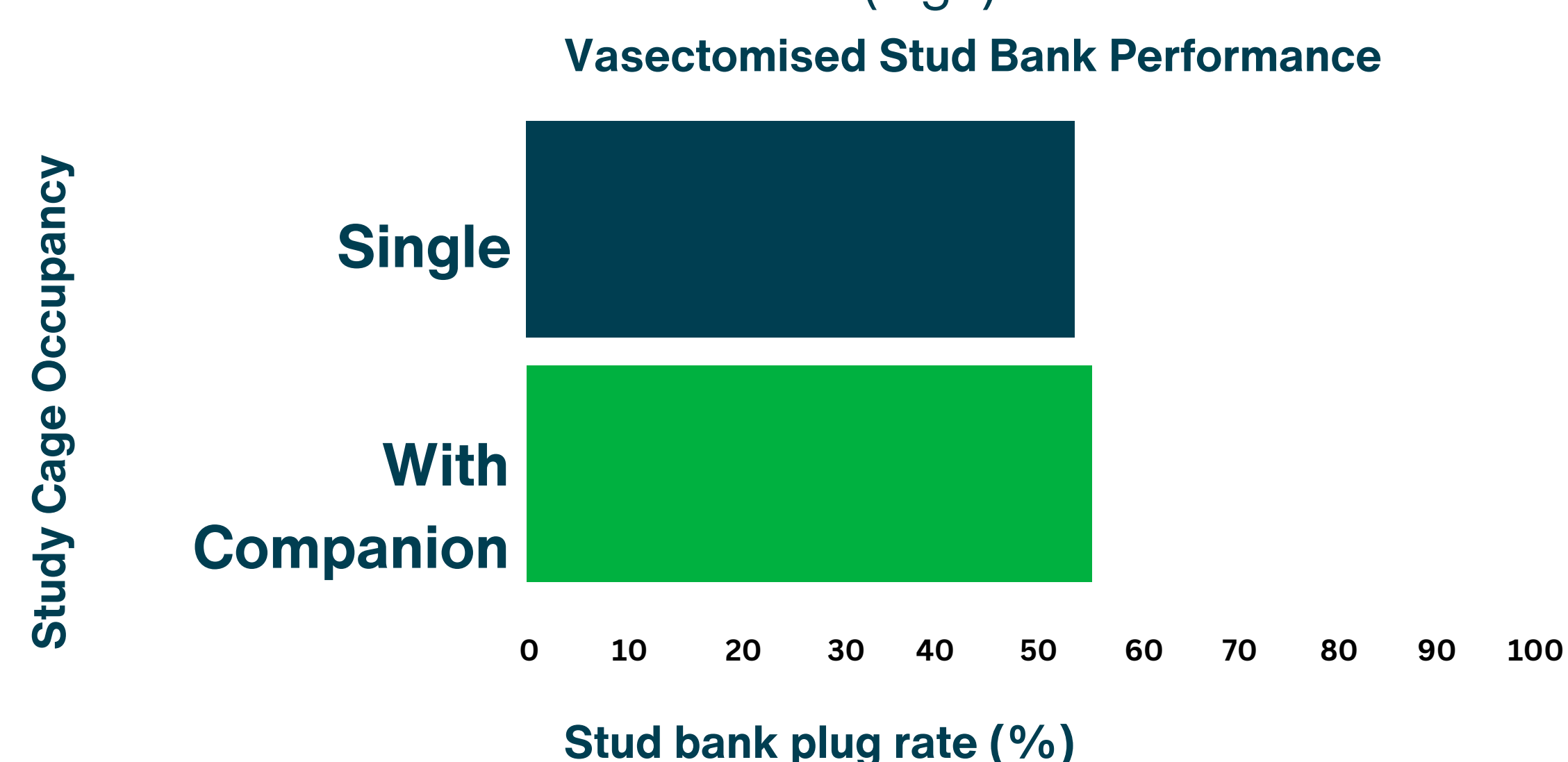
Results:

Over a 3 month trial period Non-vasectomised studs achieved a 52% plug rate whilst the studs with a companion achieved a 54% plug rate (Table 1). No significant difference in plug rate was observed (t-test, $P = .724$) and the trial was considered a success. Companions have now been introduced to the rest of the vasectomised stud bank.

(Table 1)

Stud Cage Occupancy	Total Stud Setups	Total plugs	Overall plug rate (%)
Single	990	518	52
With Companion	872	474	54

(Fig 1)



Acknowledgements

Pawel Pelczar – ISITT 3R’s Awardee 2019, PetMedix Ltd, Stuart Newman as PPLh, University of Cambridge as Establishment Licence Holder