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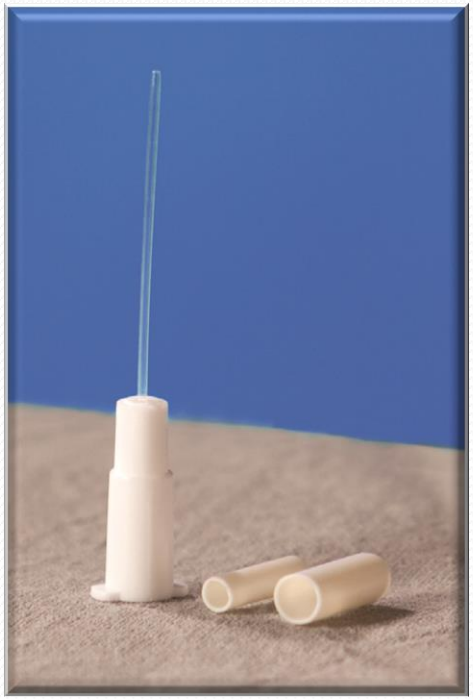
Future of Mouse Embryo Transfer: Achieving the 3Rs with the NSET device



Presented by Barbara Stone, PhD
ParaTechs Corporation
Lexington, KY, USA

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What is Embryo Transfer?

- Removal of viable embryos from a donor animal and placement of those embryos in a recipient animal
- Surgical and non-surgical methods are available
- Allows for manipulations of embryos

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Embryo Manipulations

- Embryo manipulations can be used for:
  - Generating genetically modified mice
  - Rederivation (removal of pathogens)
  - Breeding
  - Recovery of strains (including disaster or storage)
- Common embryo procedures include:
  - DNA microinjection
  - Cryopreservation of embryos
  - *In vitro* fertilization
  - Embryonic Stem (ES) cell based transgenesis
- Embryos must be transferred to a suitable recipient for development

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Why are Non-surgical Methods Important? "The 3Rs"

- Defined by Russell and Burch's 1959 book
  "The Principles of Humane Experimental Technique"
- The 3Rs are a widely accepted ethical framework for animal use in scientific experimentation
- **R**eplacement- replace an animal model
- **R**eduction- reduce the number of animals used
- **R**efinement- improved techniques (Example: NSET)

Methods for Embryo Transfer in Mice

- Oviduct Surgery
  - e0.5 embryo - e2.5 morula
- Uterine Surgery
  - e2.5 morula - e3.5 blastocyst
- Non-Surgical Embryo Transfer
  - e2.5 morula - e3.5 blastocyst

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Non-surgical embryo transfer with the NSET™ device is a 3Rs refinement technique that reduces stress in CD-1 mice

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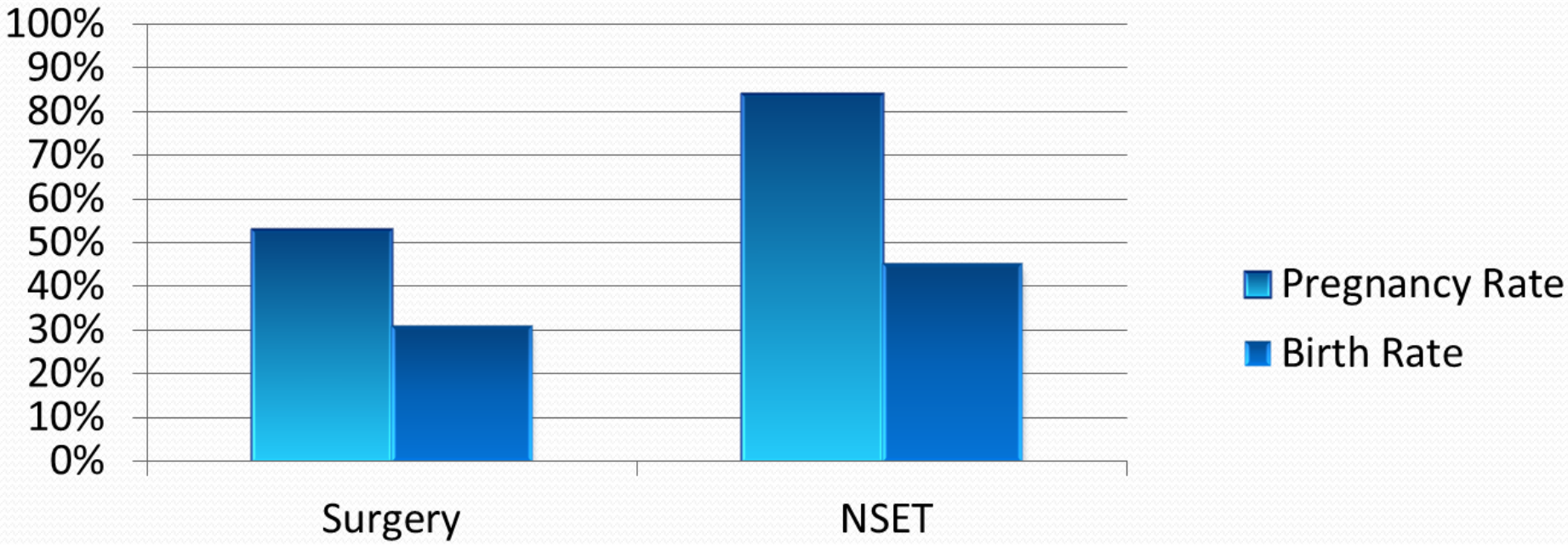
The NSET procedure is effective for embryo transfer of blastocysts



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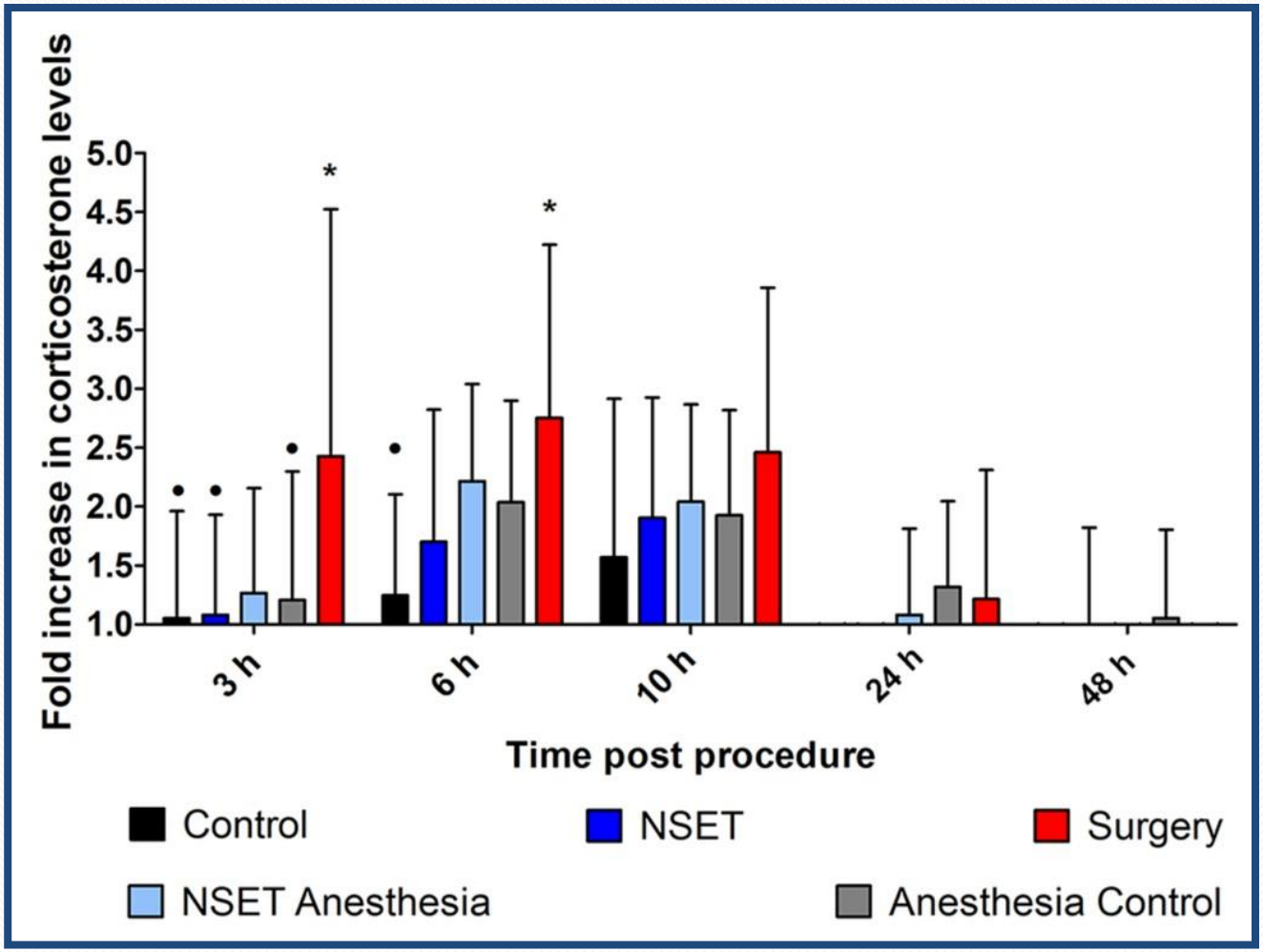
Stress hormone levels are higher in response to surgery than to insertion of the NSET device



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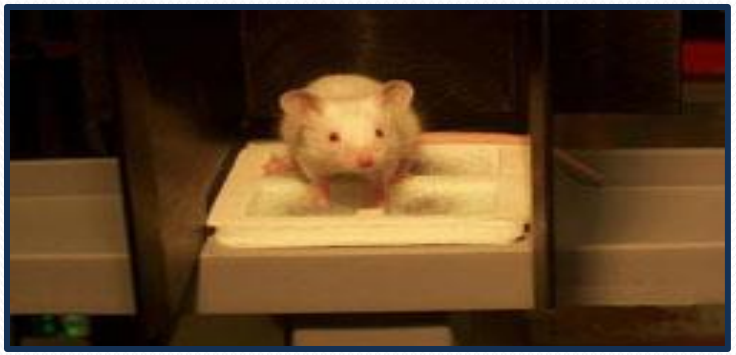
Electrocardiograms with the ECGenie™



http://www.bcm.edu/phenotyping/ecgenie.htm

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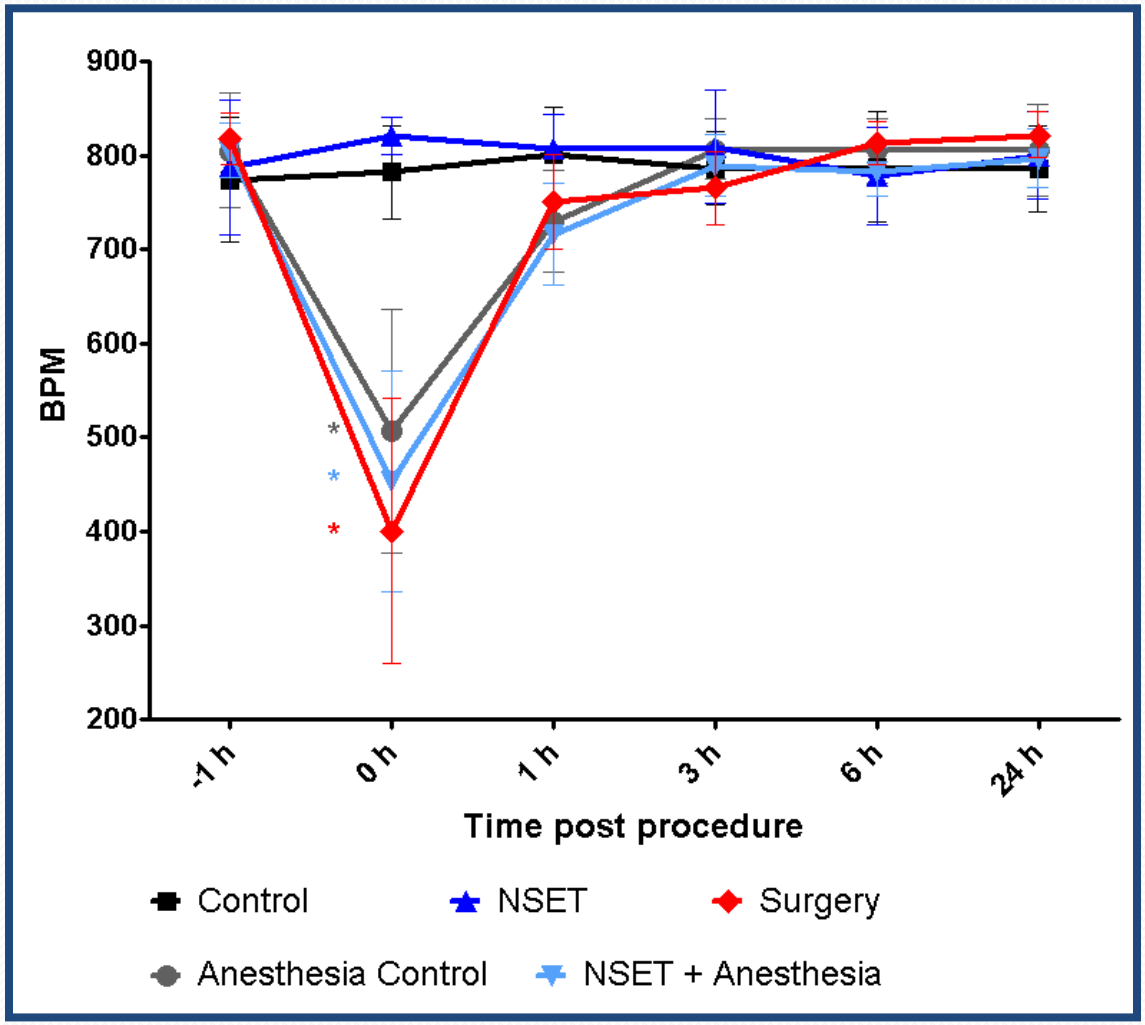
Heart rate decreases dramatically in response to anesthesia



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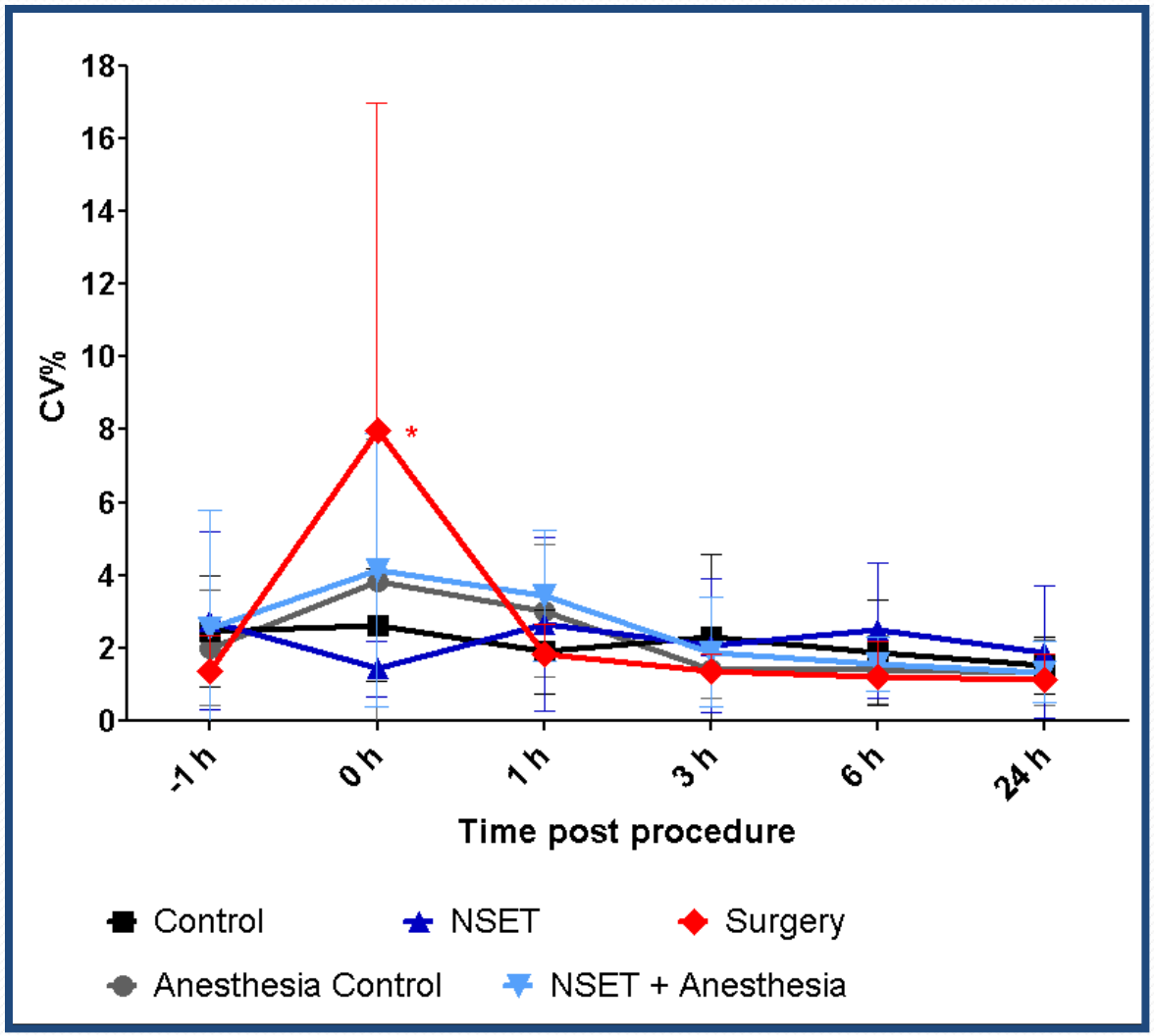
Variability in cardiac rhythm increases in response to surgery



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Conclusions

- The NSET procedure is effective for uterine transfer of blastocyst stage embryos.
- Surgery causes a significant increase in levels of the stress hormone corticosterone, whereas the NSET procedure does not.
- Anesthesia causes a 38-50% decrease in the number of heart beats per minute, whereas the NSET procedure does not alter heart beats per minute.
- Surgery increases cardiac variability by 8%, whereas anesthesia alone and the NSET procedure do not significantly increase cardiac variability.
- **Use of the NSET™ device is less stressful to mice than surgical embryo transfer.**

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Embryo Transfer Applications

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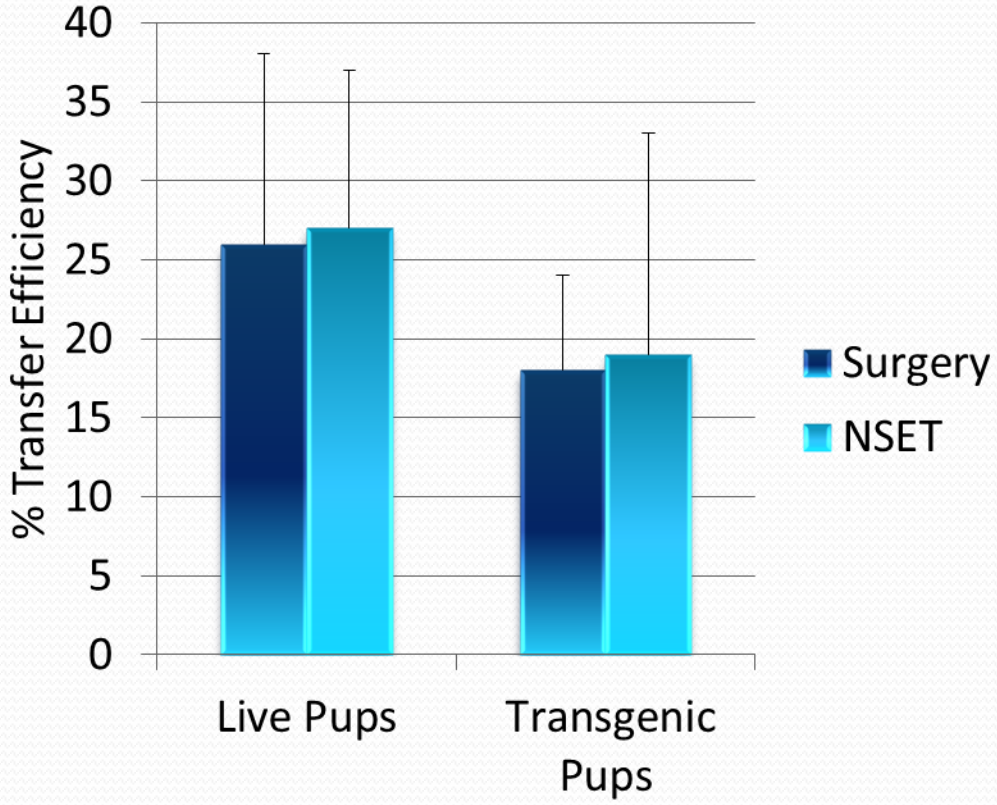
NSET of Microinjected Embryos



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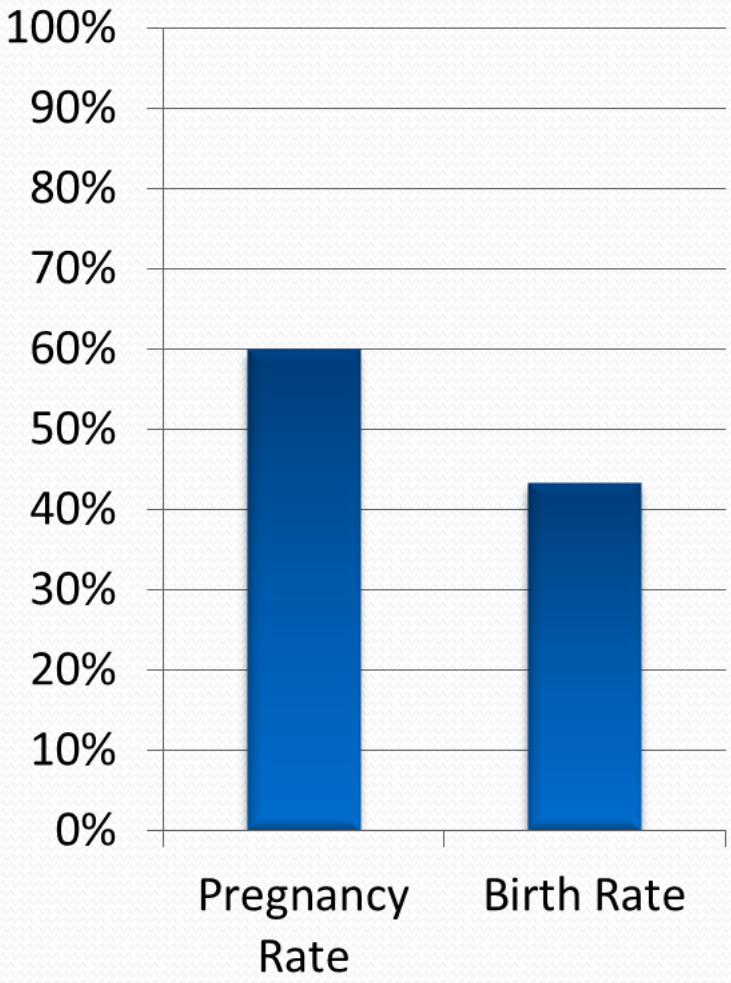
In Vitro Fertilization



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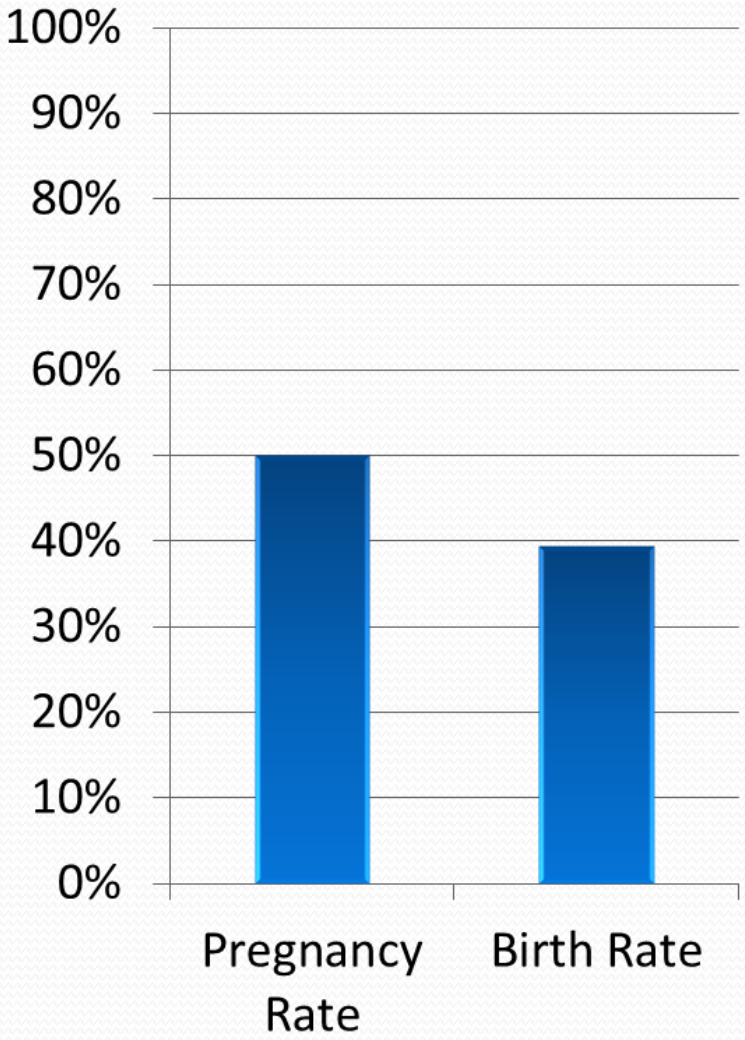
Cryopreservation



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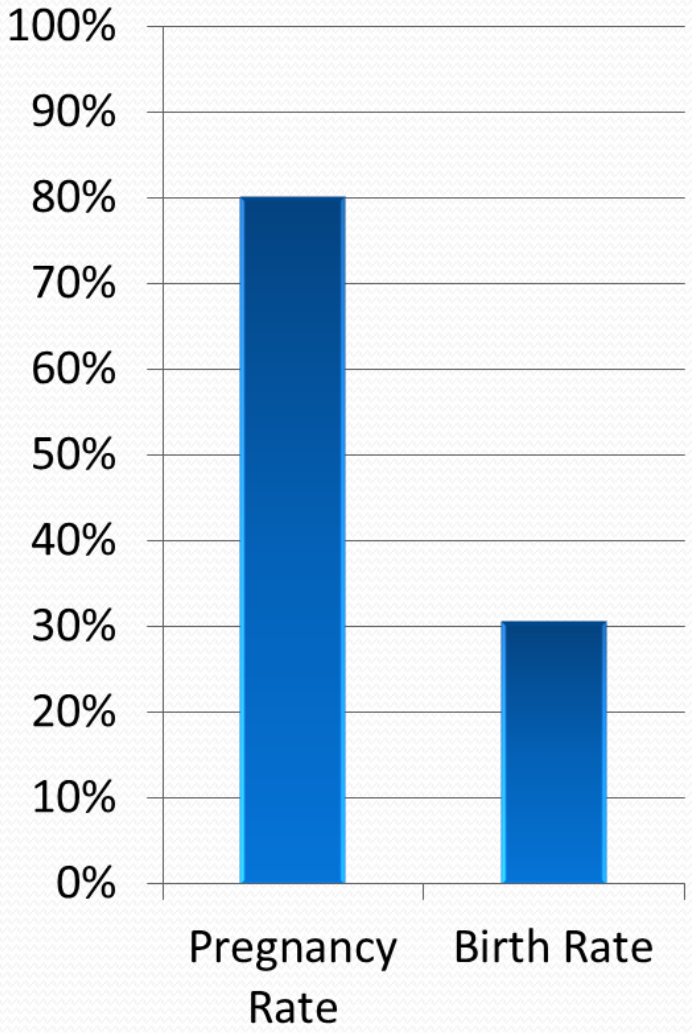
ES Cell Injections



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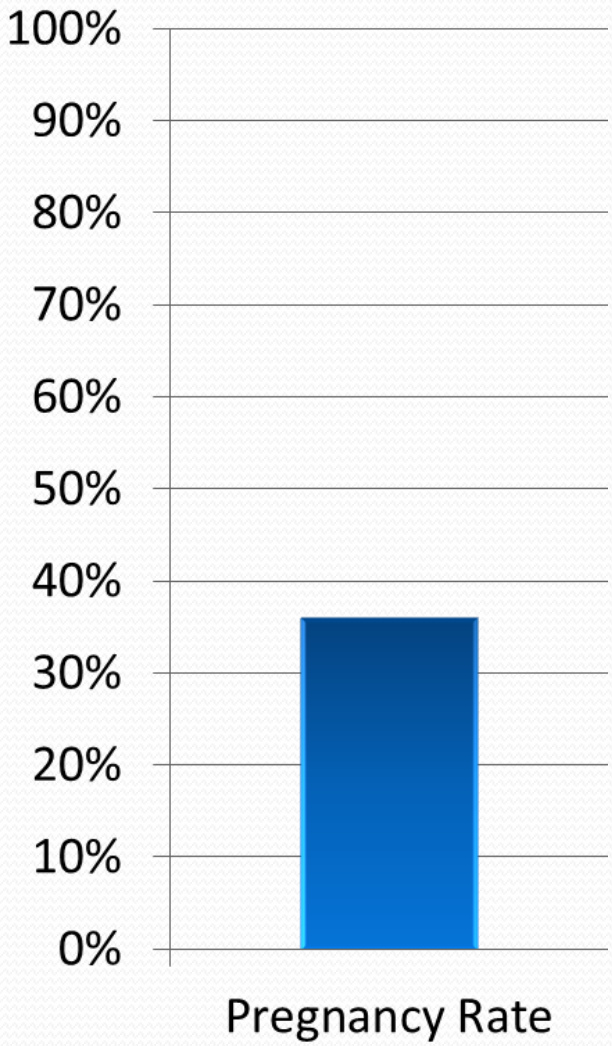
Artificial Insemination



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Conclusions

The NSET device can successfully transfer:

- Embryos after microinjection of DNA when cultured to blastocyst stage resulting in recovery of transgenic pups
- Cultured blastocysts derived from *in vitro* fertilization
- Cultured blastocysts developed from cryopreserved early-stage embryos
- ES-cell injected blastocysts resulting in recovery of chimeric pups
- Sperm during artificial insemination resulting in live births
- Pathogens such as *Chlamydia trachomatis*
- Other materials for research: concanavalin A (conA) coated Sepharose beads, drugs, etc.

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The NSET Device:

Non-Surgical Embryo Transfer Device for Mice

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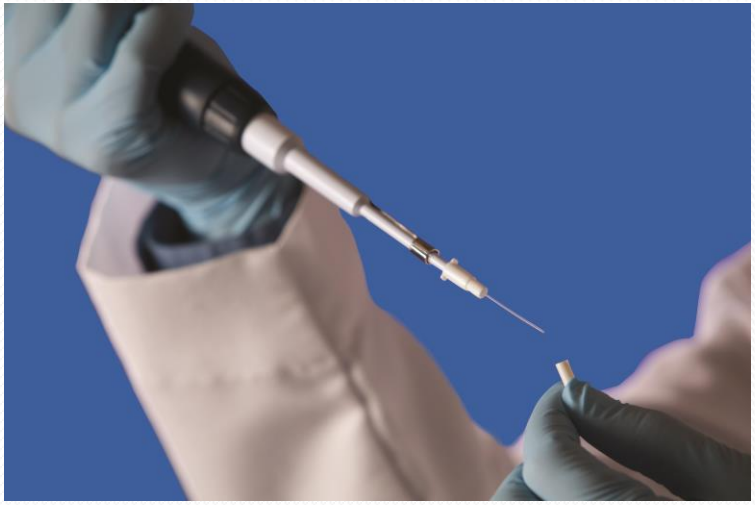
NSET Procedure

Brett Spear and Michael Green, Inventors

- Load embryos into device
- Prepare recipient mouse
- Deliver embryos

Prepare Embryos

- Attach the NSET device to a Rainin/Gilson P-2 pipette
- Load up to 20 embryos into the NSET device in 1.8 ul
- Adjust the volume to 2 ul



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Prepare Recipient Mouse

- Select a suitable female mouse, 2.5 dpc pseudopregnant
- Hold mouse in position on cage top
- Insert small speculum into vagina and remove
- Insert larger speculum into vagina
- Visually locate the cervix

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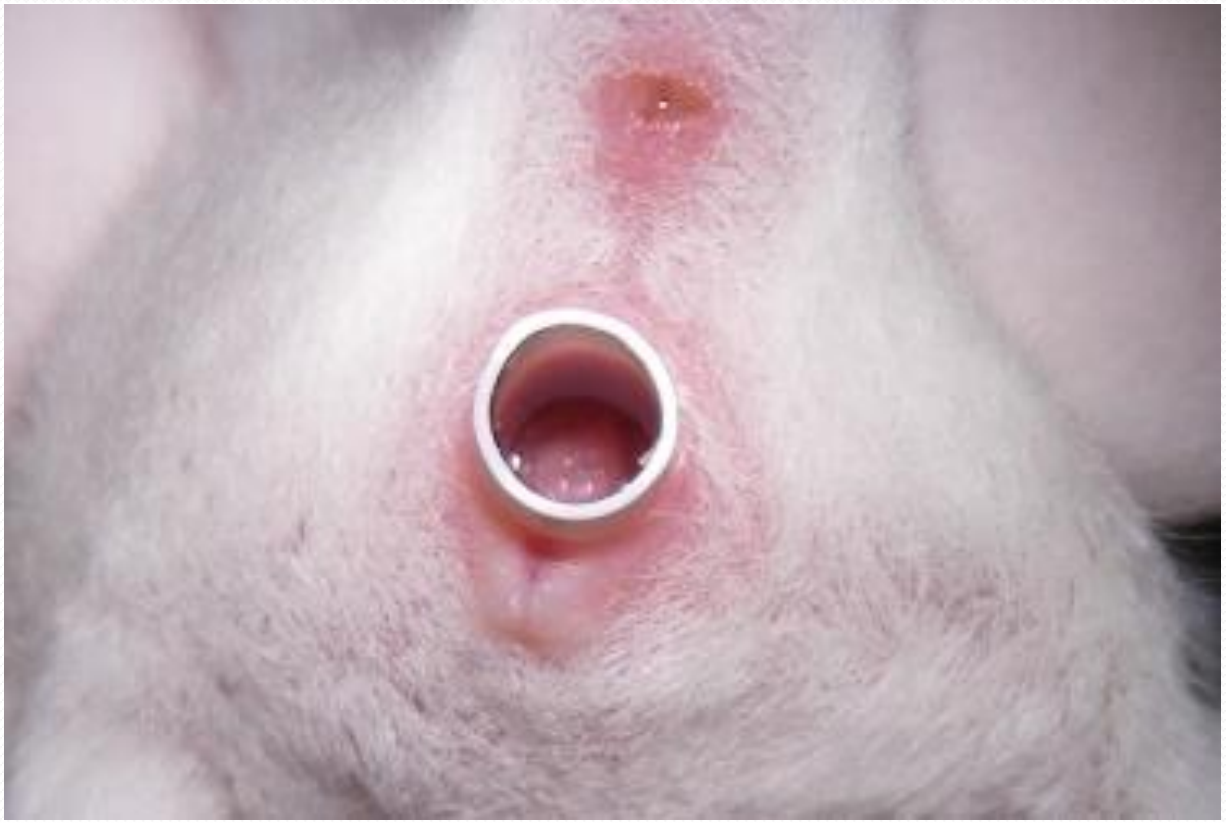
Identification of the Cervix



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NSET Video Demonstration

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NSET Video Demonstration

Please view the NSET Demonstration Video on our website www.paratechs.com

The video is an .mp4 file can be downloaded to your computer.

Complete NSET Instructions, Frequently Asked Questions, and Helpful Hints can be viewed on the website as well.

Email questions to: barbarastone@paratechs.com or info@paratechs.com

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Helpful Hints and Tips

- Remove NSET device from the uterine horn without releasing pipette plunger
- NSET device was designed as a single use device. Repeated use will clog the NSET tip with tissue which could lead to unfavorable results
- Inspection of tip after use is good practice
- Do not use lubricants
- Please make sure to read the NSET instruction sheet thoroughly before use

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Which recipient strain should you use?

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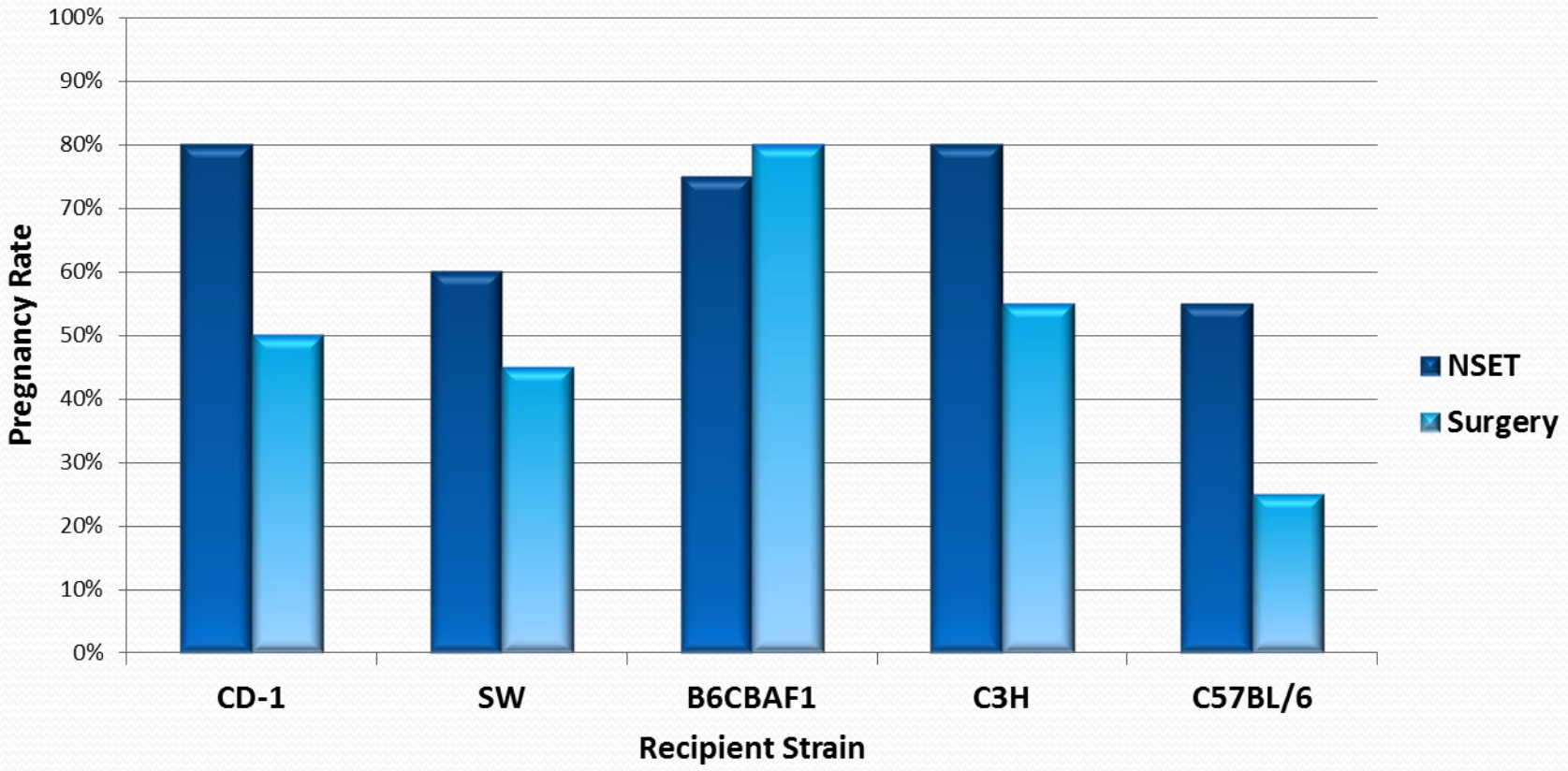
Strain dependent pregnancy rates for embryo transfer methods



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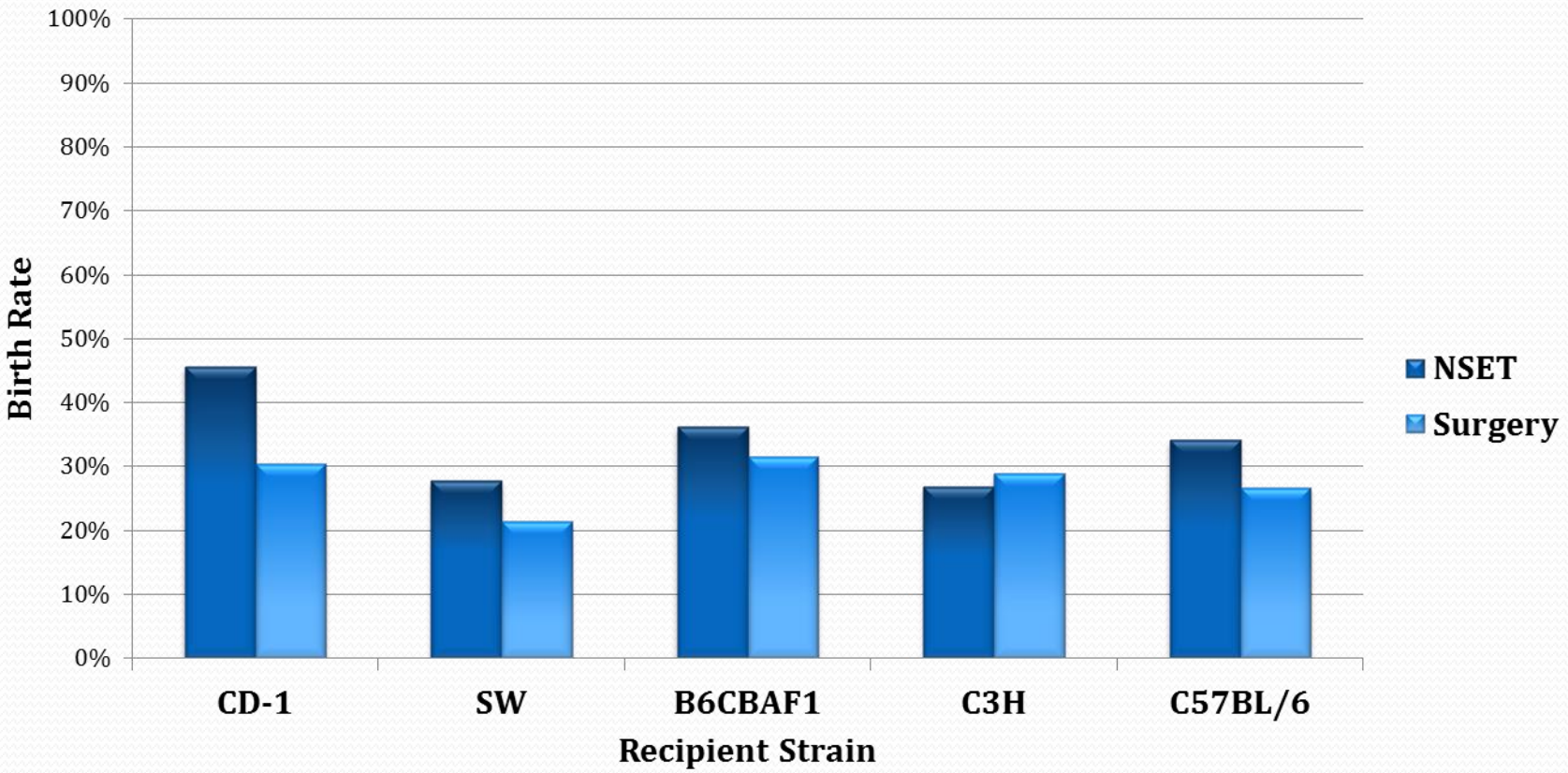
Strain dependent birth rates for embryo transfer methods



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Conclusions

- Many strains may be used as recipients for embryo transfer
- Strains are most often chosen for convenience and ease of use
- 2.5dpc CD-1 pseudopregnant females are recommended

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Advantages of the NSET procedure

- Eliminates surgical procedure
  - No pain or distress
  - No anesthesia or analgesia required
  - No post-surgical monitoring
  - Surgical training not required
- Satisfies one of the 3Rs (Refinement)
- Reduces regulatory burden
- Time-saving
- Convenience
- Cost reduction

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Acknowledgements

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Research Team

- Kendra Steele, PhD
- James Hester
- Angelika Fath-Goodin, PhD

NSET Publications

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Embryo Transfer:

- Steele KH, Hester JM, Stone BJ, Carrico KM, Spear BT, Fath-Goodin A. (2013) Non-surgical embryo transfer device (NSET) is less stressful than surgery for embryo transfer in mice. JAALAS. Jan; 52(1): 17-21.
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- Green M, Bass S, Spear B. (2009). A device for the simple and rapid transcervical transfer of mouse embryos eliminates the need for surgery and potential post-operative complications. Biotechniques. Nov; 47(5): 919-24.

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- Barrette VF, M.A. Adams, B.A. Croy. 2012. Endometrial decidualization does not trigger the blood pressure decline of normal early pregnancy in mice. Biol Reprod 86(3):66.

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Questions?

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