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Using the NSET for embryo transfer and artificial insemination in mice and rats

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Outline- *Here to make your life easier*

- Non-surgical embryo transfer in mice
- Non-surgical artificial insemination in mice
- The future of rat research

Embryo transfer

- A procedure that transfers embryos from a superovulated female and implants into another female

Who perform embryo transfer?

- Generate transgenic mice
  - A mouse gene is manipulated (deleted, repeated)
  - Mutated DNA is injected into the mouse embryo
  - Embryo is transferred to another mouse
- Rederivation (removing pathogens)
- Maintenance of strains
  - Breeding
  - Cryopreservation

Problems with embryo transfer

- Surgery
  - Technically challenging
  - Time-consuming
    - Training
    - Monitoring
  - Costly
  - Painful and stressful procedure for the rodent
    - Need for a 3 R's Refinement

Solution- NSET™

"The future of embryo transfer"

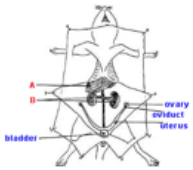
Surgical protocol

Embryo transfer uterine horn surgery

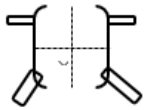
Procedure from Manipulating the mouse embryo: a laboratory manual, 3rd edition, p.268. Rewritten here by K Steele. Updated 1-29-16

I. Preoperative period (preparation):
   a. Spray all surfaces with Clidox. Let sit for up to 10 minutes.
   b. Recovery area: Adjust heating pad to level 3. Put recovery box ½ on top of heating pad and line with paper towels.
   c. Prep area
      1. Clippers out (≤ 40 size), clipper brush and paper towel ready
      2. Prepare analgesic.
   d. Surgical area
      1. Set the Cook benchtop incubator to 38.9°C.
      2. Put a sterile diaper pad on top of the Cook incubator and one right side of the incubator. Open a paper towel and place on the left side of the incubator.
      3. Prepare nose cone on Cook incubator. Prepare light source.
      4. On left side of Cook incubator, set out GenTeal eye ointment,70% ethanol, betadine scrub, sterile swabs, a sterile gauze, and analgesic.
      5. On the diaper pad to the right side of the Cook incubator, open a pack of **1)** sterile surgical equipment **2)** wound clipper and **3)** a 26G needle. *Surgical equipment includes sharp scissors, blunt forceps, two forceps with teeth, sharp forcep, serrefine clip.*
      6. Drape mouth pipette with embryo syringe over a nearby microscope.
      7. Turn bead sterilizer on. Place gauze nearby.
   e. Check equipment
      1. Check the isoflurane level within the vaporizer. The level should be halfway. If you need to refill, fill the canister through the lower right gasket using the dispense attachment.
      2. Check oxygen supply is sufficient.

II. Intraoperative period:
   a. Prepare anesthesia
      1. Confirm the isoflurane stopcock is horizontal to tube, so that the gas is supplied to the chamber not the nose cone.
      2. Twist open the oxygen canister and turn the flow meter on the oxygen tank on to level 1.5
      3. Place the animal in the induction chamber and lock down the latch
      4. Turn on isoflurane vaporizer to 3 (push white lever down)
   b. After 6 minutes, the animal will be anesthetized as indicated by lying on its side and breathing regularly.
   c. Meanwhile, prepare analgesic: 1) 0.0005 ml/ g weight working solution bup. (0.02 mg/kg animal buprenorphine), 2) 0.0004 ml/ g weight (2 mg/kg animal meloxicam).
   d. Load embryo syringe with **10**-15 healthy embryos.
   e. 1. Turn off isoflurane. Flush the chamber with 100% oxygen.
      2. Immediately open the chamber and remove the rat to the prep area.
      3. Clip fur covering the surgical side (on back, starting from top of leg).
      4. Then move rat to Cook incubator and place her nose into the nose cone.
      5. Turn the stopcock to turn nose cone on and chamber off.
      Never flush the chamber when the animal is connected to the nose cone. Turn isoflurane on to level 2.5.



   f. Apply GenTeal ointment with a sterile q-tip into each eye.
   g. Inject both analgesic SQ.
   h. Apply betadine solution to the surgical site, starting from the center and moving in a circular outward motion. Add 70% ethanol. Repeat.
   i. Using a sterile gauze, gently pinch toes to confirm the rat is anesthetized. You may be able to lower the isoflurane level.
   j. Locate the incision site.
      1. Make a lateral incision (0.5 cm) in the epidermis. Locate the fad pad.
      2. With sharp forceps, grasp a thick chunk of the dermis and make a small, longitudinal, deep incision (avoiding blood vessels) and stretch the incision with scissors.
   k. Using blunt forceps, pull out the ovarian fat pad to locate the red ovary and uterine horn. Clip the fat pad with a Serrefine clamp to keep it out of the way.
   l. Gently, wrap the embryo pipetter around your neck.
   m. Hold the uterine horn gently with blunt forceps and make a hole using a 26 G needle (bevel facing up). Use blood vessels as a guide to where you made the hole. Carefully move the needle slowly out and slide the embryo pipetter into the hole. Insert embryos, watching to see volume dispense. *If the embryos are not depositing, the pipetter opening may be blocked against the UH wall. Slowly move the pipetter up and down until the embryos dispense.*
   n. Check under the microscope that the embryos have been expelled.
   o. Unclip the Serrefine clamp and use blunt forceps to replace the fat pad and ovary into the body cavity.



   p. Close both body walls with 1 or 2 wound clips: Use sharp forceps to pull inner dermis layer up. Then use another sharp forcep to pull epidermis up around the inner dermis layer. Close with wound closure tool.
   q. Turn stopcocks to flow isoflurane towards chamber. Move rat to the recover box.

III. Postoperative period
   a. 1) Turn off the isoflurane vaporizer, 2) Flush anesthesia chamber again. 3) Check oxygen tank level and turn off tank and 4) oxygen flow meter once it has reached 0.
   b. Turn off bead sterilizer and Cook incubator.
   c. Disinfect area with Clidox. Allow to sit on surface for up to 10 mins.
   d. After an hour and once the rat is active for at least 10 minutes, place the rat in a clean cage with a *post-operative cage* card.
   e. Prepare a post-surgical monitoring log for each rat. Record Buprenorphine use in controlled substance log.

NSET protocol

NSET procedure (without anesthesia)

1. Set pipette to 1.8 µl and attach the rNSET.
2. Draw up embryos. Adjust pipette to 2.0 µl.
3. Insert rNSET speculum then embryos.

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The NSET Device:

Non-Surgical Embryo Transfer Device for Mice

NSET Procedure

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- Attach the NSET device to a Rainin P-2 pipetman (set to 1.8 µl)
- Load up to 20 embryos into the NSET device
- Adjust the volume to 2 µl to create air bubble

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Prepare Recipient Mouse

- Hold mouse in position on cage top
- Insert small speculum into vagina
- *Optionally,* replace small speculum with larger speculum into vagina to visually locate the cervix

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Perform embryo transfer

- Insert NSET through cervix and into the uterine horn
- Dispense embryos
- Remove NSET. Remove speculum.

“That’s it!?!”

- All users

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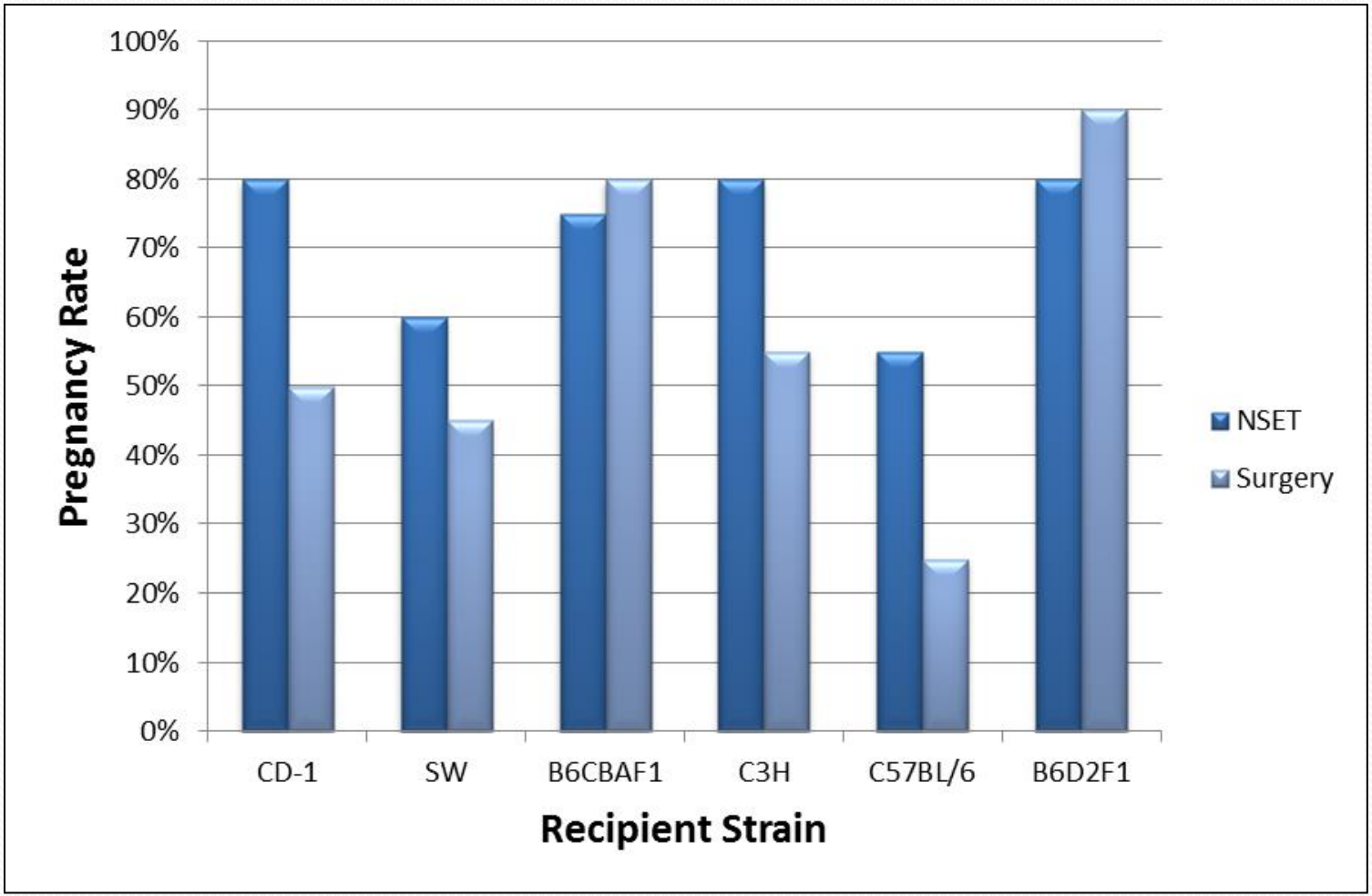
The pregnancy rate from blastocyst transfer is better or equal using the NSET compared to surgery



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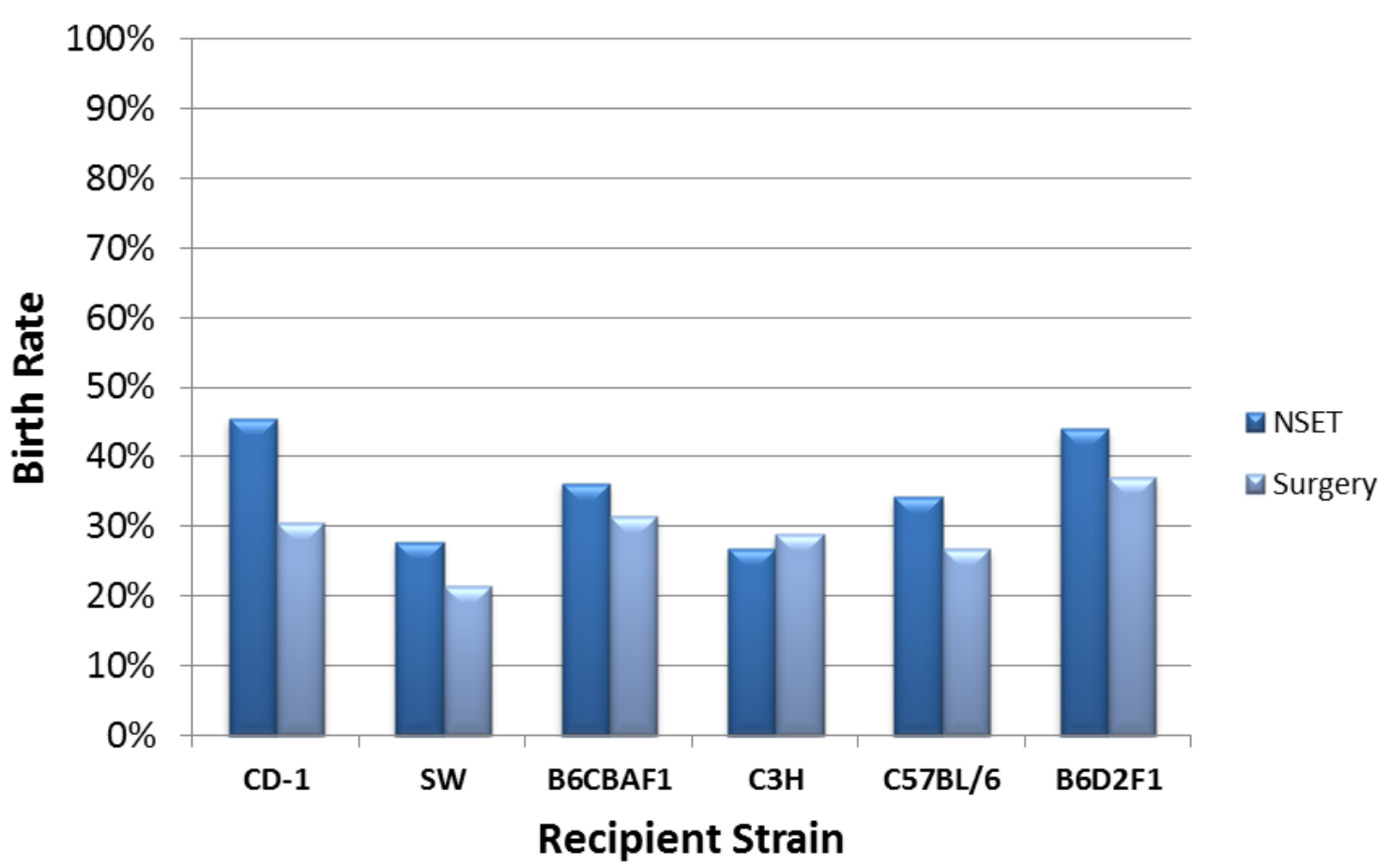
The birth rate (# pups / embryo transferred) is the similar in both procedures



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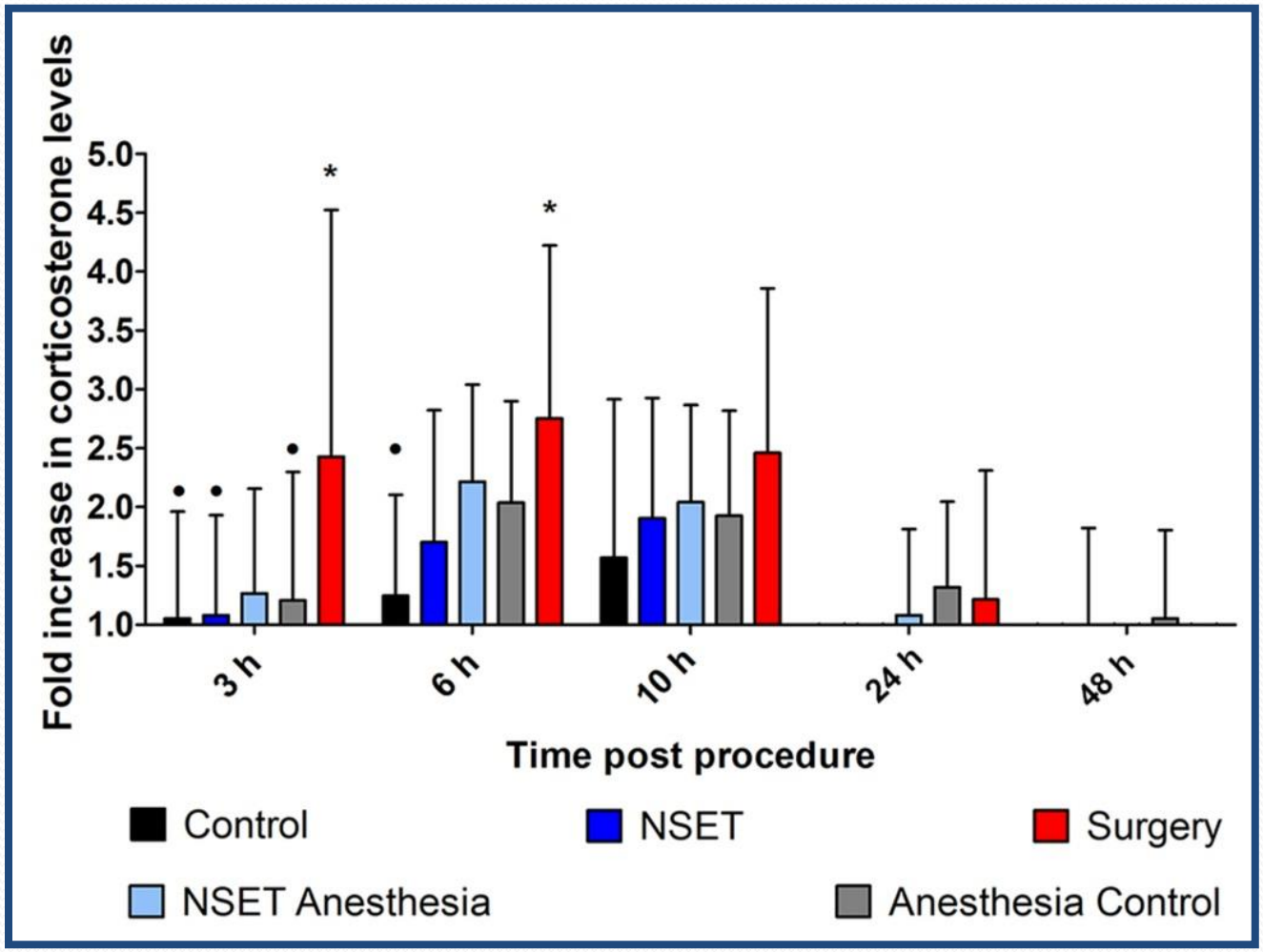
Stress hormone levels are higher in response to surgery than to insertion of the NSET device



(*) significant difference between the control group and other groups

(•) significant difference between the surgery group and other groups.

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Advantages of NSET

- Eliminates surgical procedure
  - No pain or distress
  - No anesthesia or analgesia required
  - No post-surgical monitoring
  - Surgical training not required
- Time-saving
- Reduces regulatory burden
- Reduces cost up to 75%

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Summary

- The NSET procedure is effective for uterine transfer of blastocyst stage embryos.
- Many strains may be used as recipients for embryo transfer
- Surgery causes a significant increase in levels of the stress hormone corticosterone, whereas the NSET procedure does not.
- **Use of the NSET™ device is less stressful to mice AND easier for you!**

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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
- Pathogens such as *Chlamydia trachomatis*
- Other materials for research: concanavalin A (conA) coated Sepharose beads, drugs, etc.

Artificial Insemination (AI)

A procedure when sperm is transferred to the reproductive tract of a female to make her pregnant

Removes the need for sexual intercourse

Who performs AI?

- Some transgenic mice are unable to breed, breed poorly, or the mutation affects fertility

- Recovery of cryopreserved sperm
  - Stored in case of natural disaster, infection
  - Sperm can be shipped
  - Save money on continuous breeding

Problems

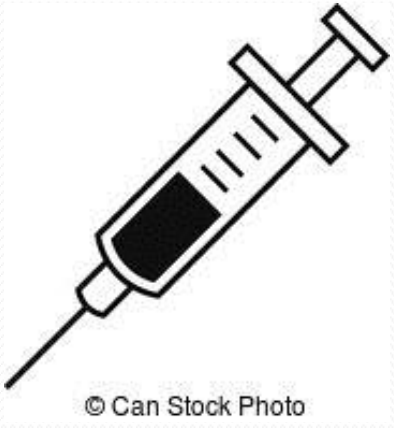
- Surgical artificial insemination
  - Requires highly skilled personal- oviduct transfers
  - Anesthesia and analgesic
  - Regulatory burden
  - Causes pain in rodent

- Non-surgical artificial insemination
  - Requires a blunt syringe needle or glass speculum
  - Daily progesterone administration
  - Inconvenient timing
    - Day 1, PMSG 1 pm Day 3, hCG 12 pm Day 4, Sperm transfer 1:30 am

The NSET solution

Stone, B. J., *et al.* 2015. Transgenic Res. **24**(4): 775-781

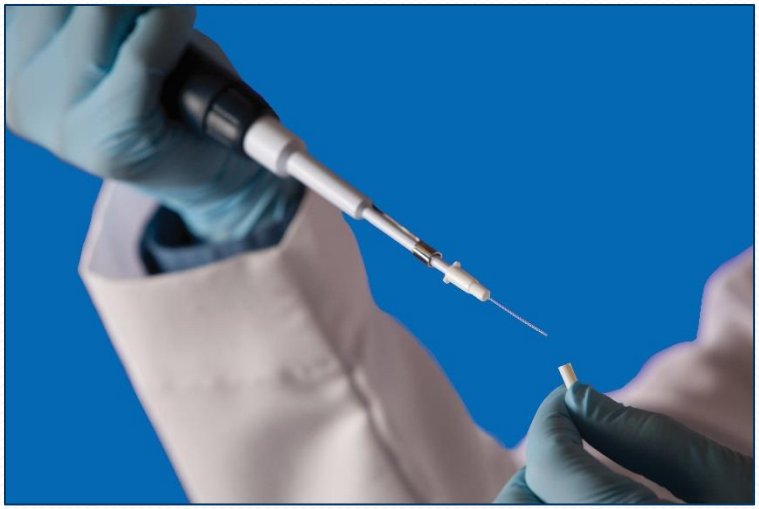
- 3 days before transfer
  - Inject 1 IU PMSG at 5:30 pm
- 1 day before transfer
  - Inject 1 IU hCG at 5:00 pm



The NSET solution

Stone, B. J., *et al.* 2015. Transgenic Res. **24**(4): 775-781

- Day of transfer
  - 8 am, Isolate sperm from the cauda epididymis and van deferens
  - Capacitate sperm 1 h in HTF
  - 9 am, Perform AI
    - Hold mouse in position on cage top
    - Insert small speculum into vagina
    - Transfer 40 µl sperm using NSET
  - Mate overnight with a vasectomized male



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Artificial Insemination in Mice

- 50% pregnancy rate
- Average litter size is 5 pups
- 100% survival rate

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A.I. Summary

- The NSET procedure
  - is effective for artificial insemination
  - replaces the painful and time-consuming surgical procedure
  - Includes a convenient schedule for hormone administration and sperm transfer

Overall conclusions

- NSET is the future of embryo transfer
  - 3 R's refinement of the surgical procedure
  - As effective as surgery
  - A *gazillion* times easier for the user

- NSET is also used for artificial insemination

Future Directions

It's time for the rats…

The Rat Model

- Rat technology was previously limited
  - Rat embryonic stem cell technology was introduced in 2008
- But the rat is physiologically closer to humans than mice
  - Models of human disease
    - Autoimmune diseases
    - HIV
    - Arthritis
    - Hypertension
- Now we can create banks of transgenic rats

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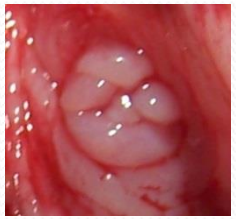
Rats reproductive considerations

- Basic anatomy is similar to mice except:
  - Vagina is longer and wider
  - Cervix has two openings
  - Uterine horn is much longer



- Physiology of pregnancy is different
  - Timing of embryo development is longer
  - Mouse blastocysts are e3.5 (2.5 dpc recipients)
  - Rat blastocysts are e4.5 (3.5 dpc recipients)
  - Cervix of rat is open at 2.5 dpc (same as mice)

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First Success of the rNSET Device

rNSET transfer of blastocysts at 2.5dpc results in pups

Acknowledgements

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Barbara Stone Coles

Angelika Fath-Goodin

James Hester

Margo Landis

Office of the Director, National Institutes of Health under Award Numbers 1R43RR025737-01A1, 2R44RR025737-02, 8R44OD010958-03, 3R44OD010958-03S1, R43RR030684-01, and 2R44OD018231-02A1

Kentucky Cabinet for Economic Development, Office of Commercialization and Innovation KSTC-184-512-11-115

Kentucky Science and Technology Corporation 184-512-10-096

Kentucky Science and Engineering Foundation KSEF-3407-RDE-018

NIH National Institutes of Health
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