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

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Introduction

Non-surgical embryo transfer (NSET) can be used to transfer blastocysts to recipient mice during the generation of transgenic mice, after cryopreservation of embryos, after *in-vitro* fertilization, and during rederivation of mouse strains. Non-surgical embryo transfer is a refinement (one of the 3Rs) of the surgical embryo transfer procedure. The NSET™ device is a small tapered catheter that attaches to a P2 pipette and deposits embryos directly into the mouse's uterine horn. Use of an NSET™ device should substantially reduce pain, suffering, and recovery time of mice as the surgical procedure is replaced with an alternative method. No anesthesia is required for non-surgical embryo transfer and the mice require no post-procedure recovery. Therefore, we hypothesized that use of the NSET™ device is less stressful than surgery for the mouse. We compared the stress responses of pseudopregnant CD-1 mice (2.5 days post coitum) following uterine embryo transfer surgery and non-surgical embryo transfer procedures using the NSET™ device. ELISA analysis was used to quantify levels of fecal corticosterone, a biomarker of stress. Electrocardiograms were performed using a non-invasive device, the ECGenie by Mouse Specifics, Inc., to measure cardiac indicators of stress.

NSET™ Advantages

- Simple procedure
- Does not require anesthesia or an analgesia
- Requires less time to
  - Prepare the mouse
  - Perform the procedure
  - Monitor the mouse for post-surgical complications
- Requires less equipment
- Uses less space
- Reduces costs up to 75%

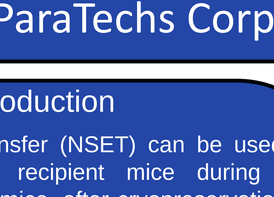
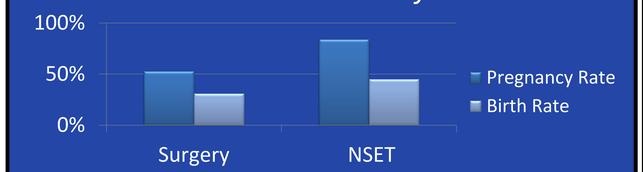


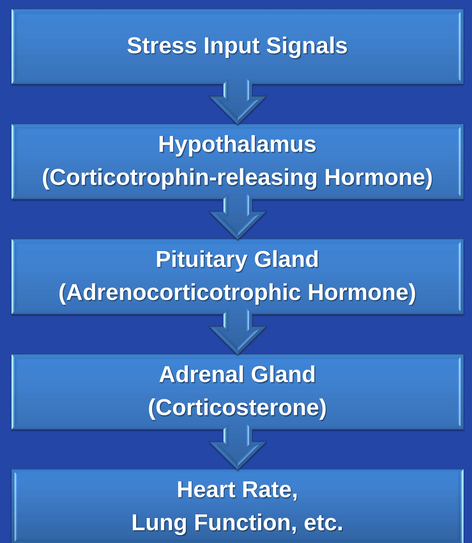
Photo courtesy of Marcelo F.G. Nogueira (UNESP)

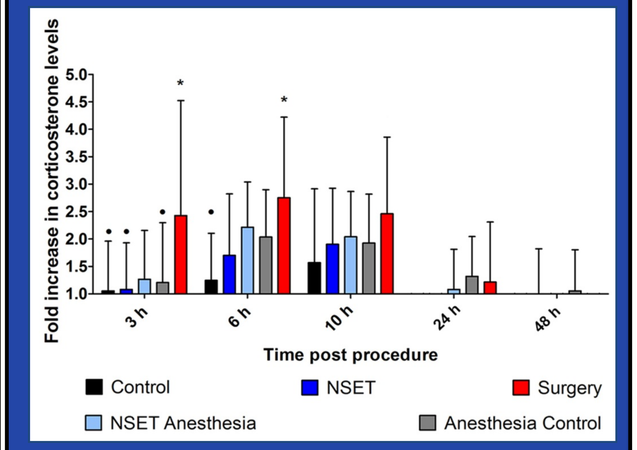
The NSET procedure is effective for embryo transfer of blastocysts.



Surgical and NSET procedures are both effective for blastocyst transfer to the uterine horn. Blastocysts (e3.5) were transferred to the uterine horn of 2.5 dpc pseudopregnant female CD-1 mice. Pregnancy rate (% of females becoming pregnant after embryo transfer) and birth rate (% of pups per embryos transferred to females that became pregnant) are compared.

Fecal corticosterone levels are higher in response to surgery than to insertion of the NSET™ device.





Fecal corticosterone levels are significantly increased in mice 3-6h after surgery. Fecal samples were collected from pseudopregnant mice (2.5 dpc) assigned to five experimental treatment groups (N=15). Corticosterone levels were measured by ELISA assay (Cayman Chemical Co., Ann Arbor, MI) according to the manufacturer's directions.

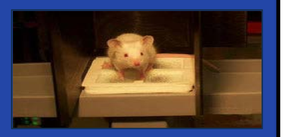
(*) represents a significant difference between the control group and other groups.

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

Electrocardiograms with the ECGenie™

Electrocardiograms are recordings of the electrical currents that pass through the heart and signify the activity associated with each heart beat. The non-invasive ECGenie instrument was used to record electrocardiograms from mice without restraint or a surgical implant. Readings were made by placing the mouse on a sensory pad outfitted with three monitoring electrodes. The cardiac activity was measured passively through the paws. Time necessary to record an acceptable cardiac signal depended on the mobility of the mouse and averaged between 5 - 8 minutes.

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



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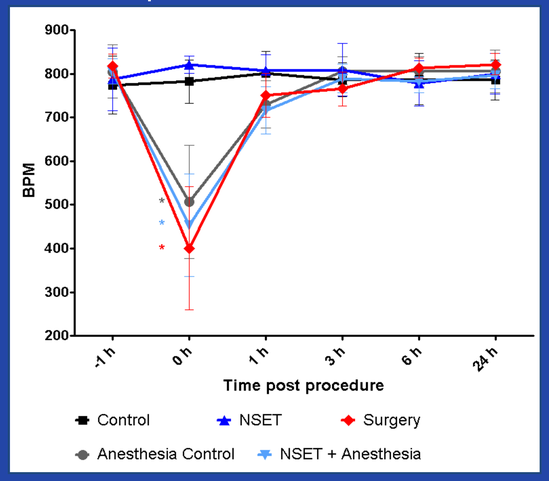
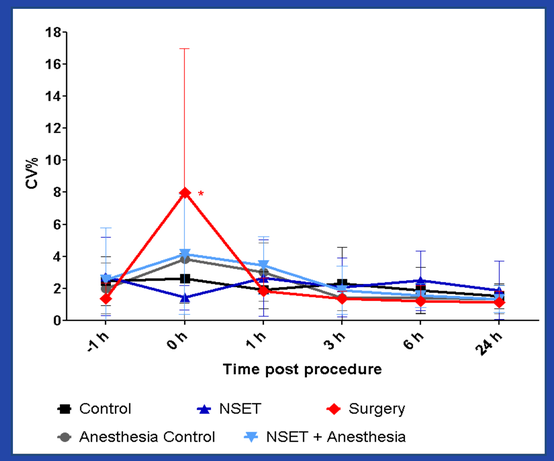
Heart rate decreases dramatically in response to anesthesia.



Heart rate in beats per minute (BPM) are graphed at various hours (h) post procedure for 2.5 dpc pseudopregnant CD-1 mice. Data was analyzed by Mouse Specifics using EzCG analysis software. Data presented are means and standard deviations (N=11). Statistical significance ($P \leq 0.05$) for the comparison of the control group versus the experimental groups is represented by an asterisk (*).

Variability in cardiac rhythm increases in response to surgery.



Variability in beat-to-beat rhythm of the heart is measured as the coefficient of variance (CV%) and is graphed at various hours (h) post procedure for 2.5 dpc pseudopregnant CD-1 mice. The data was analyzed by Mouse Specifics using EzCG analysis software. Data presented are means and standard deviations (N=11). Statistical significance ($P \leq 0.05$) for the comparison of the control group versus the experimental groups is represented by an asterisk (*).

Conclusions

- The NSET procedure is effective for uterine transfer of blastocyst stage embryos.
- Surgery causes a significant increase in fecal corticosterone levels, 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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