Non-Surgical Embryo Transfer in Rats; A 3Rs Refinement for Assisted Reproductive Technology

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Introduction

A 3Rs concern for rat research models is the requirement for surgical transfer of embryos after targeted mutagenesis or as an assisted reproduction technique. In order to eliminate the need for surgical embryo transfer, rat non-surgical embryo transfer (rNSET) technology has been developed. NSET technology (US patent No. 9,615,903 B2) supports humane production, maintenance, preservation, and transport of existing and newly developed rat strains. Here we describe rNSET device design and the first protocol for successful non-surgical transfer of blastocysts resulting in pups. To determine if rNSET use is stressful to female rats, the stress responses of non-surgical and surgical embryo transfer recipients are compared by fecal corticosterone production and weight gain analysis. The procedures for non-surgical and surgical methods of transfer are compared for birth rate and embryo transfer efficiency.

rNSET Device Design

Anatomical measurements of the rat reproductive tract were compared in two rat strains with significant differences in body size (>50 grams); Sprague-Dawley (SD) and Fischer 344 (F344) rats. The average weight of SD females was 209.54 grams ± 21.13, while the F344 rats weighed 157.29 grams ± 12.96. No statistical difference was observed between the two strains of rats for various aspects of reproductive tract measurements (Figure 1). The similarities between the two strains in the length of the uterine horns and the distance from the vaginal opening to the uterine horns are essential parameters for rNSET device design. This enabled development of a single device for use in both strains. Several prototypes (Figure 3) were tested for ease of use, depth of insertion (Figure 2), and safety. One device was then chosen for embryo transfer testing.

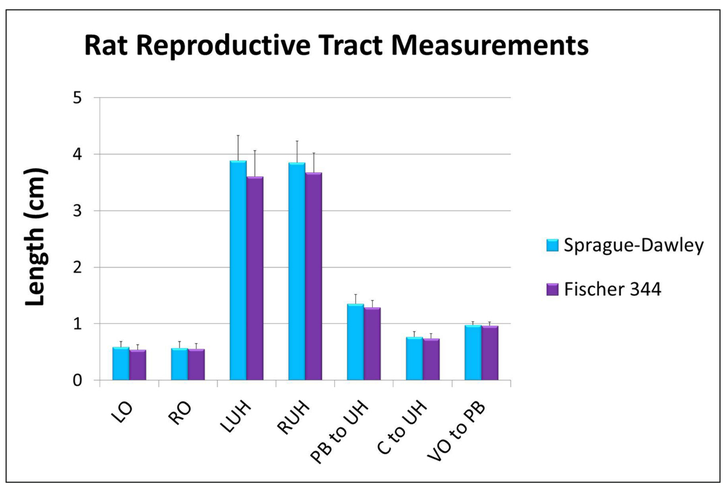

Figure 1. Reproductive Tract Measurements. A comparison of the lengths of the left ovary (LO), right ovary (RO), left uterine horn (LUH), right uterine horn (RUH), end of the pubic bone nearest the cervix to the uterine horn (PB to UH), cervix to the uterine horn (C to UH), and vaginal opening to the end of the pubic bone nearest the cervix (VO-PB) in Sprague-Dawley (blue bars) and Fisher 344 (red bars) rats. Error bars represent standard deviation.

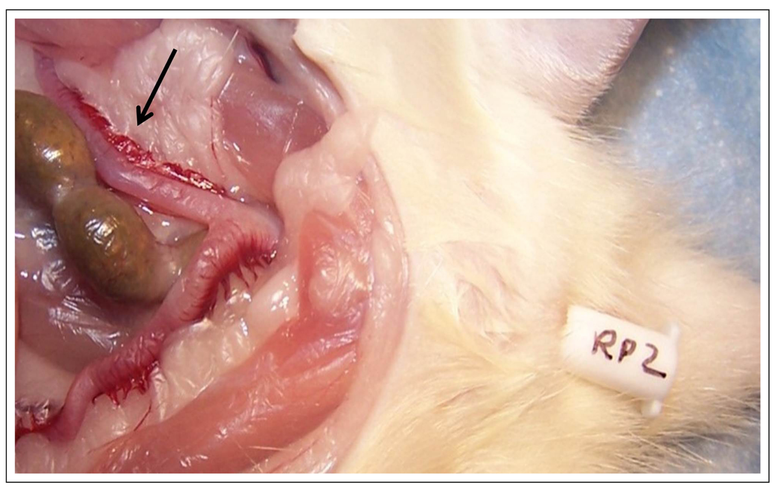

Figure 2. rNSET prototype insertion. Rat NSET prototype 2 (RP2) is shown inserted into the left uterine horn of a SD rat. A small bend in the uterine horn shows were the tip of the catheter is positioned (arrow). The speculum was inserted vaginally, the rNSET catheter was inserted past the cervix and into the uterine horn, and dissections were performed.

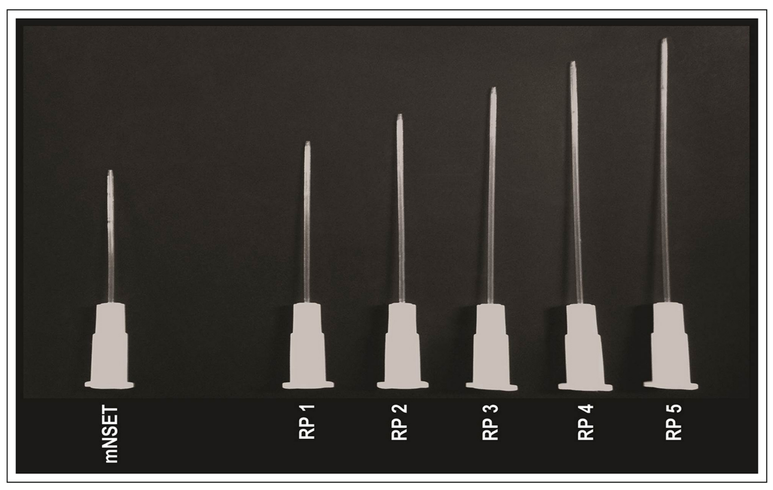

Figure 3. Manufactured rat NSET prototypes. The NSET device tip is made from FEP (Teflon) and the hub is molded from polyethylene. The hub is designed to fit securely on the end of a P-2 pipette. The tip is tapered to facilitate passage through the cervix, with the length of the catheter optimized for embryo delivery through the cervix to the uterine horn. The catheter is comprised of flexible tubing so it readily passes through the cervix without damage to the uterine wall. The mouse NSET[1,2] (mNSET) and rNSET prototypes (RP1-RP5) are shown for comparison.

Stress Response Analysis

The use of a non-surgical procedure as a replacement for a surgical procedure provides clear advantages to the welfare of animals in research. However, to determine if a non-surgical procedure produced a more stressful situation than a surgical procedure (with appropriate anesthesia and analgesia), the stress response for two non-invasive stress biomarkers was measured. For these studies, fecal corticosterone levels (Figure 4) and weight loss (data not shown) were compared between rats after surgical or non-surgical embryo transfer. No significant differences were observed for experimental groups experiencing no procedure, anesthesia only, non-surgical embryo transfer with or without anesthesia, or surgical embryo transfer for either stress biomarker.

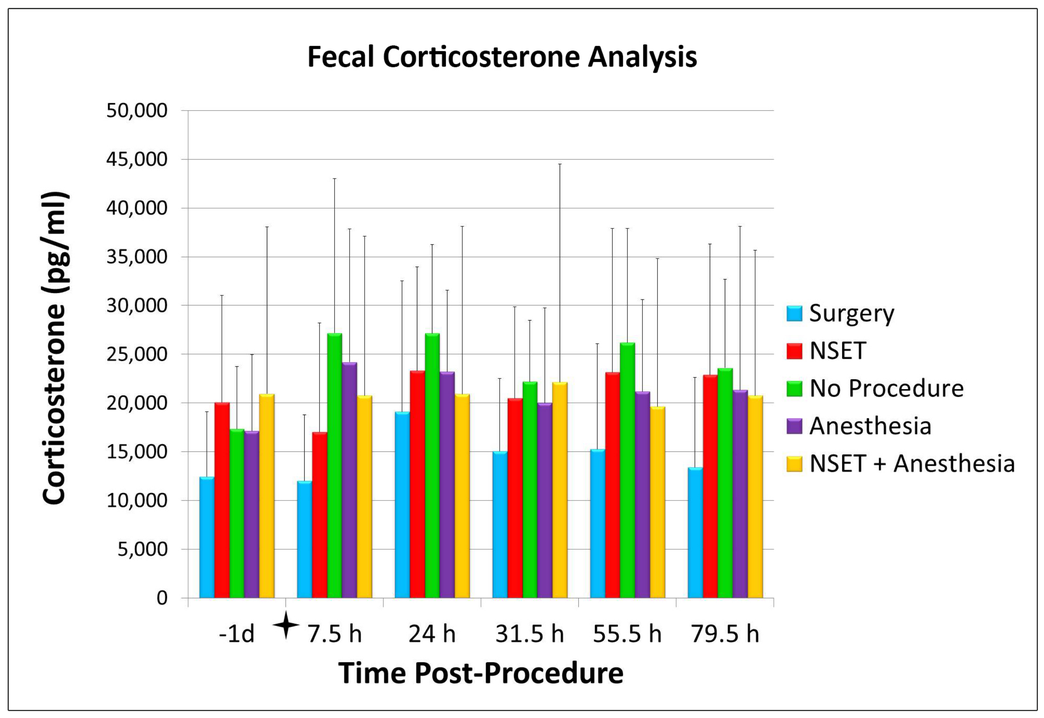

Figure 4. Fecal corticosterone level comparison. For all groups (SD, N=26, >8 weeks old), fecal pellets were collected 1 day prior to the procedure; and at 7.5, 24, 31.5, 55.5, and 79.5 hours post procedure. The time of procedure is indicated (✦). The "no procedure" and "anesthesia" control groups did not have pseudopregnancy induced and were otherwise handled for collection the same as other groups. Pseudopregnant rats at 2.5days post coitum (dpc) were used as recipients for all embryo transfer experiments. Recipient rats were estrus synchronized with 40µg luteinizing hormone releasing hormone agonist (LHRHa) four days prior to mating with a vasectomized SD male. For the surgery group, embryos were transferred by standard uterine transfer under isoflurane with administration of 2mg/kg meloxicam and 0.02mg/kg buprenorphine. All groups receiving anesthesia received the same isoflurane/ meloxicam/buprenorphine treatment for a standard 15 minute procedure. For recipients in the NSET groups, a speculum was first inserted into the vagina. The rNSET device was inserted through the speculum and the cervix. After embryos were delivered, the device and speculum were removed.

Non-Surgical Embryo Transfer with the rNSET

Comparison embryo transfer studies were performed with blastocysts from SD females transferred by surgical or rNSET technique to 3.5dpc pseudopregnant SD or F344 recipients (Table 1). For the rNSET procedure, the cervix of the female was dilated with 1-2IU oxytocin. The rat speculum was inserted vaginally. The rNSET device catheter was inserted through the speculum, past the cervix, and into the uterine horn where the embryos were deposited. The rNSET device and speculum were then removed. For surgical embryo transfer, a dorsal midline incision was made, through which the ovary and uterine horn were exposed. A small incision in the uterine horn was created and embryos were delivered by pipette. The ovary and uterine horn were returned to the abdominal cavity and the incision closed with suture and wound clips. The efficiency of transfer (live births/embryos transferred) was similar for rNSET and surgery.

Table 1. Embryo Transfer Efficiency

Procedure	Rat Strain	Transfers	# Embryos Transferred		Litter Size		Pregnancy Rate	Transfer Efficiency
			Range	Average	Range	Average		
NSET	SD	20	20-30	25.95	4-16	8.12	90%	31%
Surgery	SD	20	20-30	24.5	3-15	6.44	60%	26%
NSET	SD	15	12-19	14.4	1-10	5.5	73%	40%
Surgery	SD	13	11-19	15.2	2-11	6.3	62%	37%
NSET	F344	14	11-19	14.1	1-10	5.3	93%	38%
Surgery	F344	14	12-17	14.7	2-8	5.5	73%	38%

SD rat obtained from a non-surgical embryo transfer using a rNSET device. Photo courtesy of Margo Landis.

Conclusion

- The rNSET device use does not induce a stress response in embryo transfer recipients
- Non-surgical embryo transfer is as efficient as surgery for transfer of blastocysts in Sprague-Dawley and Fischer 344 rats
- The rNSET provides an efficient 3Rs alternative to surgical embryo transfer

References

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This research was supported by the Office of the Director of the National Institutes of Health under Award Numbers R43/44OD018231. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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