Successful use of the NSET™ device for non-surgical transfer of blastocysts after *in vitro* fertilization, cryopreservation, or ES-cell injection and sperm transfer for artificial insemination.

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Introduction

Non-surgical embryo transfer (NSET) using the NSET™ device is effective for transfer of blastocysts to the uterine horn of pseudopregnant recipient female mice and is an alternative to traditional surgical embryo transfer. The NSET device is a tapered Teflon catheter attached to a specially designed hub that allows the device to fit on the end of a P-2 pipette for precise liquid delivery. Once embryos are loaded into the device, the catheter passes through the vagina and traverses the cervix to deposit embryos into the uterine horn of a recipient mouse. The process is fast, does not require anesthesia or analgesia, and post-procedure recovery is not necessary. Use of the NSET device for transfer of blastocysts derived from *in vitro* fertilization (IVF), recovered from cryopreservation of fertilized oocytes, and after injection of embryonic stem (ES) cells is described. An artificial insemination technique using the NSET device for intra-uterine sperm delivery is also described.

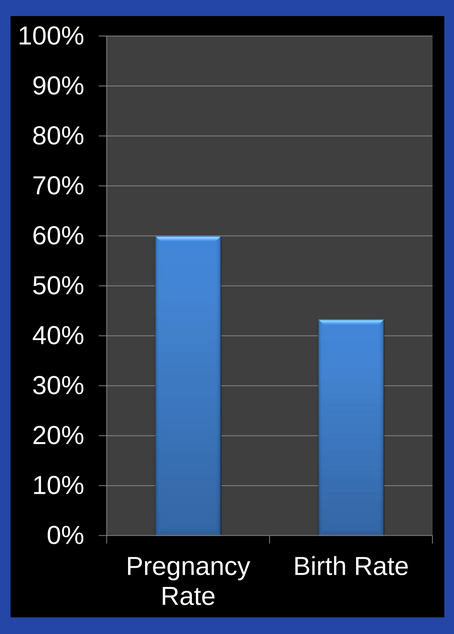
Embryo Transfer

NSET™ vs. Surgery

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

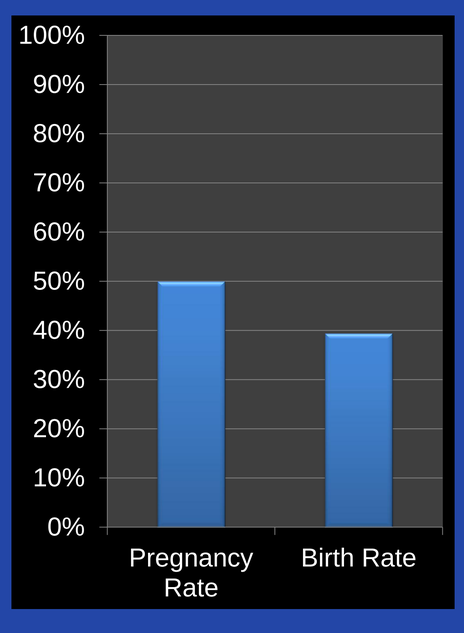
http://surgery.wustl.edu

In Vitro Fertilization



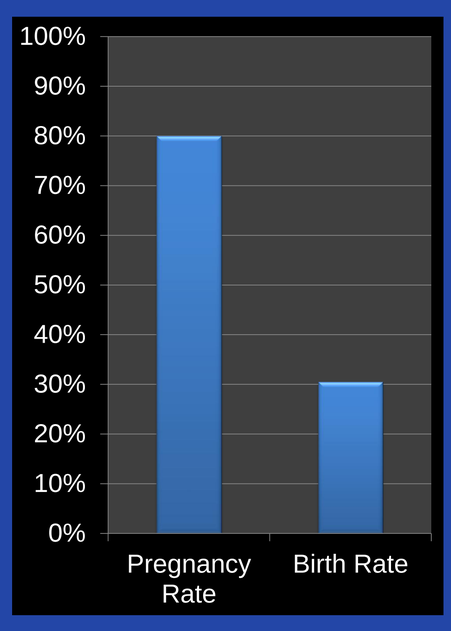
IVF of fresh oocytes from B6C3F1 female mice and cryopreserved sperm from B6C3F1 males was performed. Fertilized embryos were cultured in KSOM to blastocyst stage and transferred to 2.5 days post coitum (dpc) pseudopregnant CD-1 recipient females (N=20) using the NSET device. Transfer of IVF-derived blastocysts resulted in a pregnancy rate of 60% and a birth rate of 43% (pups / embryo transferred in females becoming pregnant). Average litter size was 7.3 pups.

Cryopreservation



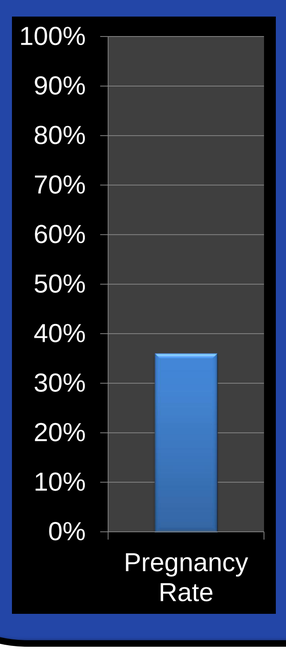
Cryopreserved B6C3F2 1-cell embryos were cultured to blastocyst stage in KSOM media and transferred to 2.5 dpc pseudopregnant CD-1 recipient females (N=16) using the NSET device. Transfer of previously cryopreserved embryos cultured to blastocyst stage resulted in a pregnancy rate of 50% and a birth rate of 39%. The average litter size was 7.8 pups.

ES Cell Injections



C57BL/6 blastocysts were injected with JM8A3.N1 ES cells and transferred to 2.5 dpc pseudopregnant CByB6F1/J recipients (N=25). The pregnancy rate from NSET transfers of ES cell-injected blastocysts was 80% with a birth rate of 31%. The total rate of chimerism (# of chimeras / # of pups surviving to wean) was 54%. Data courtesy of Peter Kutny and The Microinjection Service at the Jackson Laboratories.

Artificial Insemination



Artificial insemination of superovulated CD-1 female mice with B6C3F1 sperm transferred using the NSET device led to the delivery of live pups. 5IU PMSG were injected IP at 8:30pm three days before sperm delivery and 5IU hCG were injected IP at 8pm one day before sperm delivery into female CD-1 mice. Intra-uterine delivery of 40µl of fresh, capacitated sperm from B6C3F1 males to CD-1 female recipients using the NSET device occurred at 9am. Females were immediately paired with vasectomized CD-1 males overnight. A pregnancy rate of 36% was obtained (N=19).

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Litter size after artificial insemination ranged from 2-9 pups. Thirteen pups survived to weaning. Shown below is a 5 week old female resulting from artificial insemination using the NSET device for sperm transfer.

Conclusions

- The NSET device can successfully transfer cultured blastocysts derived from *in vitro* fertilization and cultured blastocysts developed from cryopreserved early-stage embryos.
- The NSET device can successfully transfer ES-cell injected blastocysts resulting in recovery of chimeric pups.
- The NSET device can successfully transfer sperm for artificial insemination resulting in live births.

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