Refinements to Infusion Systems for Improved Animal Welfare on Rat Fertility, Embryo/Fetal, and Pre/Postnatal Development Studies



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ABSTRACT

Continuous Intravenous (IV) infusion is a challenging route of administration on developmental and reproductive studies in the rat. Dosing duration varies from 12 days on embryo/fetal development studies to up to 9 weeks on male fertility studies. Traditional rodent infusion systems include subcutaneously exteriorized femoral vein catheters with tethers secured to jackets that require frequent adjustments during the study. Additional measures taken to protect catheters from damage include the use of stainless-steel mesh reinforced jackets during cohabitation as well as restriction of cohabitation to when room lights are on instead of the traditional method of overnight breeding. A pilot study was conducted to evaluate the feasibility of using femoral vein catheters and transcutaneous buttons with magnetic connections, including vendor-prototype magnetic tethers with aluminum sleeves. Twelve male and 12 female rats, surgically implanted by the vendor with femoral vein catheters bonded to transcutaneous buttons in the scapular area, were procured. Ten males were dosed with 0.9% sterile saline via continuous IV infusion at a rate of 2 mL/kg/hour for 28 days prior to mating and continuing during mating (to naïve untethered females) until the day prior to euthanasia (total of 64 days). 10 females were similarly dosed for 14 days prior to mating and continuing during mating (to naïve untethered males) and gestation until lactation day 20. Clinical observations, body weights, food consumption, estrous cyclicity, reproductive performance, parturition, litter viability and survival, and macroscopic findings were evaluated in addition to catheter placement, patency, and dose syringe weights. Clinical observations included mild irritation at the exteriorization site that was transient and did not impact overall animal health. In general, all animals gained weight during the study. Maternal and paternal endpoints, including reproductive performance, were unaffected and within the range of laboratory historical control data. In addition, no remarkable findings were noted for F1 offspring. All catheters were patent at study termination and the use of transcutaneous buttons with magnetic connections proved successful in this study. This technique provided an improvement to animal welfare and study efficiency as it eliminated the use of jackets and the system updates significantly reduced the need for infusion system repairs during the course of the study.



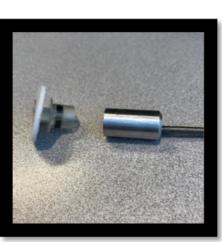




Figure 1: Magnetic tether with protective aluminum sleeve (comparison to standard tether); connection with transcutaneous button





Figure 2: Tethered dam with neonate pups

Figure 3: Tethered dam with pups near end of dosing

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MATERIALS AND METHODS

- □ 12 Adult Crl:CD(SD) male and 12 Adult female rats (Charles River Laboratories, Raleigh, NC); surgically implanted by supplier with femoral vein catheters bonded to transcutaneous buttons in the scapular area.
- ☐ Animals were single housed, identified by microchip and maintained under standard environmental/food/water conditions.
- ☐ Transcutaneous button was equipped with a magnetic tether connection (in lieu of traditional tethers secured with jackets).
- □ Vendor-prototype magnetic tethers with aluminum sleeves (Figure 1) were designed to protect the plastic portion of the connections from chewing (especially during co-housing during the breeding period).
- □ Dosing Regimen: Continuous intravenous infusion (0.9% sodium chloride for injection, 2 mL/kg/hour);
 - Males 28 days prior to mating and continuing during mating (to naïve untethered females) until the day prior to euthanasia (total of 64 days).
 - Females 14 days prior to mating and continuing during mating (to naïve untethered males) and gestation until Lactation Day 20.
- Endpoints evaluated: Clinical observations, body weights, food consumption, estrous cyclicity, reproductive performance, parturition, litter viability and survival, and macroscopic findings. Catheter placement, patency, and dose syringe weights were also collected.

3 RESULTS

- ☐ All males survived to scheduled necropsy
- ☐ All males were successfully dosed for the duration of the study.
- ☐ With the exception of 3 females, all females were successfully dosed for the duration of the study.
 - One female was found dead prior to mating, the cause of death was undetermined. Its catheter was in place at necropsy.
 - One female was found dead on Gestation Day 18 during a system repair. A thrombus was the suspected cause of death. Its catheter was in place at necropsy.
 - One female had total litter loss on Lactation Day 0. This female was noted with impaired use of hindlimbs prior to euthanasia.
 Its catheter tip was not found in place at necropsy and was suspected to be the cause of the impairment.
- ☐ During the mating period, the modified tethers with aluminum sleeves allowed the animals to be paired overnight with no damage to the tethers.
- ☐ Clinical observations included transient mild irritation at the exteriorization site with no impact on animal health.
- □ All F₀ animals gained weight during the study. Maternal and paternal endpoints, including reproductive performance, were unaffected and within the range of laboratory historical control data. All catheters were patent at study termination.
- ☐ In the F₁ offspring, no remarkable findings were noted. The presence of the litter had no impact on dam infusion system integrity.

3 RESULTS (cont'd)

Reproductive Performance (with untethered naive mate)	Button-Implanted Data	CRL HC Mean (Range)
Male Mating Index (%)	90	98.9 (92.0–100)
Male Fertility Index (%)	100	96.8 (88.0–100)
Male Pregnancy Index (%)	90	95.5 (84.0–100)
Male Pre-Coital Interval (days)	4.3	2.8 (1.8–4.4)
Female Mating Index (%)	100	99.3 (92.0–100)
Female Fertility Index (%) ^b	100	97.0 (84.2–100)
Female Pregnancy Index (%)°	100	96.3 (80.0–100)
Female Pre-Coital Interval (days)	4.4	2.8 (3.6–5.1)
Parameter Evaluated	Button-Implanted Dam/Litter Data	CRL HC Mean (Range)
Gestion Length (Days)	Button-Implanted Dam/Litter Data 21.9	CRL HC Mean (Range) 21.8 (21.3–22.1)
Gestion Length (Days)	21.9	21.8 (21.3–22.1)
Gestion Length (Days) Number Live Pups	21.9 13	21.8 (21.3–22.1) 14.1 (11.1–16.4)
Gestion Length (Days) Number Live Pups Live Birth Index (%)	21.9 13 100	21.8 (21.3–22.1) 14.1 (11.1–16.4) 98.8 (96.1–100)
Gestion Length (Days) Number Live Pups Live Birth Index (%) Live Male Pups/Litter	21.9 13 100 43.34	21.8 (21.3–22.1) 14.1 (11.1–16.4) 98.8 (96.1–100) 49.1 (41.2–56.6)
Gestion Length (Days) Number Live Pups Live Birth Index (%) Live Male Pups/Litter Mean Post-implant Loss/Litter	21.9 13 100 43.34 1.638	21.8 (21.3–22.1) 14.1 (11.1–16.4) 98.8 (96.1–100) 49.1 (41.2–56.6) 0.8 (0.3–1.7)

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CONCLUSION

- ☐ The use of transcutaneous buttons and modified magnetic tethers improved animal welfare by eliminating the use of jackets (and subsequent jacket induced irritation) traditionally used for animals with surgically implanted catheters.
- ☐ The prototype tether with protective aluminum sleeve served as an improvement over stainless-steel mesh reinforced jackets during cohabitation.
- ☐ Traditional overnight breeding was feasible with this approach, which eliminated the additional labor requirements for daily separation and repairing of males and females during the mating period.
- ☐ The reduction of infusion system repairs and the elimination of jacket adjustments during the course of the study greatly improved efficiency.
- ☐ All catheters were patent at study termination and the use of transcutaneous buttons with modified magnetic tethers proved successful in this study and were an improvement over jacketed mating and dosing.

