Published in final edited form as:

Ethology. 2012 December 1; 118(12): 1133–1139. doi:10.1111/eth.12016.

Re-feeding food-deprived male meadow voles affects the sperm allocation of their rival males

Ashlee A. Vaughn¹⁾, Javier delBarco-Trillo²⁾, and Michael H. Ferkin¹⁾

¹⁾Department of Biological Sciences, The University of Memphis, Ellington Hall, Memphis TN 38152, USA

²⁾Reproductive Ecology and Biology Group, Museo Nacional de Ciencias Naturales, Consejo Superior de Investigaciones Científicas, José Gutiérrez Abascal 2, Madrid 28006, Spain

Abstract

An individual's nutritional status affects the manner in which same- and opposite-sex conspecifics respond to that individual, which may affect their fitness. Male meadow voles, *Microtus pennsylvanicus*, increase their sperm allocation if they encounter the scent mark of an unfamiliar male that is not nutritionally challenged. If, however, the scent mark comes from a male that has been food deprived for 24 hours, stud male voles do not increase their sperm allocation. Food deprived males may be viewed as being lower quality and a reduced risk of sperm competition by rival males. We hypothesized that stud males in promiscuous mating systems tailor their sperm allocations depending on whether rival males have been food deprived and then re-fed. We predicted that newly re-fed males will be considered a strong risk of sperm competition because of the potentially high fitness and survival costs associated with food deprivation in males, and that they will cause stud males to increase their sperm allocation. Our results, however, showed that the recovery period from 24 hours of food deprivation was a relatively slow process. It took between 96 hours and 336 hours of re-feeding male scent donors that were food deprived for 24 hours to induce stud males to increase their sperm allocation to levels comparable to when scent donors were not food deprived. Stud male voles may be conserving the amount of sperm allocated until the male scent donors have recovered from food deprivation and subsequent re-feeding.

Keywords

food deprivation; meadow voles; odors; re-feeding; sperm allocation

Introduction

Males in a number of taxa tailor their sperm allocation prudently to increase the likelihood of siring a litter when they are exposed to a risk of sperm competition (Parker & Pizzari 2010). Male meadow voles, *Microtus pennsylvanicus*, and male rats, *Rattus norvegicus*, faced with a high risk of sperm competition from a rival male will increase the amount of sperm in their ejaculate (delBarco-Trillo & Ferkin 2004, 2006; Pound & Gage 2004). Recent work has also shown that male voles adjust their sperm allocation when faced with differing risks of sperm competition from rival males. Male voles allocate a smaller number of sperm in their ejaculate when they were exposed to the scent marks of rival males that had been food deprived for 24 hours than when they were exposed to the scent marks of males that had not been food deprived (Vaughn et al. 2008).

*Corresponding author: ashlee.a.vaughn@gmail.com; phone: 901-678-3509.

This finding suggests that male voles may perceive food-deprived males as being a lower risk of sperm competition relative to males that were not food deprived. Males not perceived as being a high risk of sperm competition do not cause rival males to increase their sperm allocation. Rival male voles could tailor their sperm allocation and increase their own reproductive success (Vaughn et al. 2008; Parker & Pizzari 2010). In addition, the nutritional status of male voles could affect their mating opportunities. Nutritionally-challenged male voles produce scent marks that are less attractive than those produced by male voles that were not nutritionally challenged (Ferkin et al. 1997). Female voles are more likely to display proceptive behaviors towards males that were not nutritionally challenged as compared to males that were challenged (Hobbs et al. 2008, 2011a, 2011b). Several studies on a variety of taxa have shown that food deprived or food restricted males have lower mating and reproductive success than do males that were not so treated (Kodric-Brown 1989; Proctor 1992; Samuel et al. 1999; Plath et al. 2005; Fisher & Rosenthal 2006). Thus, food-deprived male voles may be perceived by rival males as being low risks of sperm competition because the former males likely have few mating opportunities.

Free-living populations of terrestrial mammals often face periods of low food availability (Bronson 1989). Such a situation may also occur in free-living meadow voles, in areas such as transitional grasslands (Madison 1980; Getz 1985). Male meadow voles wander through large home ranges that encompass the territories of one or more females. Across these territories, food is distributed in patches that vary in quality and abundance (Batzli 1985; Bergeron & Jodoin 1987; Bergeron et al. 1990). In searching for females, male voles may move into areas that have poor quality forage. The potential exists for acute food deprivation to occur before the male leaves that female's territory and moves into another territory in which food is more plentiful. As males wander through these territories they deposit scent marks to indicate their presence in the area as well as aspects of their condition, including their nutritional status (Ferkin et al. 1997, 2004; Roberts 2007). These male scent donors may have been food deprived or are in the process of re-feeding. In either case, the nutritional status of these males may affect the behavior of the resident females and nearby male conspecifics. The responses of these males and females may in turn affect their mating and reproductive success, as well as affecting a male's perception of a rival as a risk of sperm competition. Male voles will likely tailor their sperm allocation when they face rival males that differ in their perceived risk of sperm competition (Vaughn et al. 2008). Thus, after a period of re-feeding, previously food-deprived males will also be perceived by rival males as a higher risk of sperm competition than they were before re-feeding.

In the present study, we determined how long male voles must be re-fed in order to cause rival males to perceive them as high risks of sperm competition by inducing their rivals to increase their sperm allocation when exposed to their scent marks. We hypothesized male meadow voles that were food deprived for 24 h recover quickly from this nutritional challenge and after a short period of re-feeding induce their rivals to increase their sperm allocation. This hypothesis is based on the natural history of male meadow voles. The fitness of male voles depends on their mating with multiple females and siring many offspring (Boonstra et al. 1993; Berteaux et al. 1999). However, male meadow voles are short-lived mammals (Getz 1960), and are not likely to over-winter or survive to mate again during the next breeding season (Tamarin 1985). Consequently, a male will benefit by recovering his reproductive potential as quickly as possible after a period of food deprivation, which will make other males to perceive him again as a high risk of sperm competition and thus increase their sperm investment in his presence.

Methods

Animals

The meadow voles used in this study were offspring of field-caught animals, captured in Pennsylvania, and housed in the animal care facility at The University of Memphis. These voles were housed in a room that was controlled for temperature, approximately 23° C, and on a 14:10 h light-dark cycle to simulate day length during breeding season; lights were turned on a 0700 CST. Meadow voles were weaned at 19 d of age and kept with littermates until they were 34 d old. They were then housed singly in clear polycarbonate cages (27 × 16.5 × 12.5 cm). Cages contained hardwood shaving as bedding and cotton for nesting material. Food and water were provided *ad libitum*, except for scent mark donors in the food-deprived and re-fed conditions (see below). The male scent donors and stud males were similar in age (between 6–9 mo-old), weight (within 5 g), and sexual experience (having sired a litter). The scent donors were unrelated and unfamiliar to the stud male and female. We followed Animal Care Protocols 505 and 609, which were approved by the IACUC at The University of Memphis. We adhered to the ‘Guidelines for the use of animals in research’ as published in *Animal Behaviour* (1991, 41, 183–186) and the laws of the country where the research was conducted. The experiment began in June 2010 and was completed in January 2012.

Female meadow voles are induced ovulators and do not undergo regular estrous cycles (Milligan 1982; Keller 1985). However, adult female voles born and reared in long photoperiod are sexually receptive and capable of mating with multiple partners when they reach sexual maturity (Meek & Lee 1993). Long-photoperiod meadow voles also respond preferentially to the scent marks of opposite-sex conspecifics compared to those of same-sex conspecifics (Ferkin & Johnston 1995). The female voles used in this study were 6–9 mo-old, adult, and sexually receptive, and sexually experienced having previously delivered a litter; none of the females were pregnant or lactating at the beginning of the experiment.

Treatment Groups

Seventy male and 70 female meadow voles were used in this study, with 10 different males and 10 different females used in a control group and each of the six different treatment groups. In the control group, a female and a male vole were allowed to mate without the scent marks of a nearby male being present. In the six treatment groups, female and male voles were paired in the presence of the scent mark of a male that had continuous access to food (1M), the scent mark of a male that was food deprived for 24 h (24-FD), the scent mark of a male that was food-deprived for 24 h and then re-fed for 24 h (24-RF), the scent mark of a male that was food-deprived for 24 h and then re-fed for 72 h (72-RF), or the scent mark of a male that was food-deprived for 24 h and then re-fed for 96 h (96-RF). After measuring the sperm allocation of stud males in these treatments, we then decided to add another group. We allowed a female and a male vole to mate in the presence of the scent mark of a male that was food-deprived for 24 h and then re-fed for 336 h (336-RF), and measured the sperm allocation of stud males exposed to these re-fed males. All tests were conducted between 0700–1000 CST. Scent donors and stud males within each treatment group were from different litters.

Testing Procedure

We used males from the treatment groups as scent donors, following methods detailed in previous studies (Ferkin & Johnston 1995; Pierce et al. 2005; Vaughn et al. 2008). In the control condition, fresh distilled water was placed on a sterile cotton applicator and the applicator was rubbed for five seconds on the center portion of a clean, glass microscope slide (7.5 cm × 2.5 cm). In the 1M, 24-FD, 24-RF, 72-RF, 96-RF, and 336-RF conditions the

anogenital area of the male scent donor was rubbed against the center portion of a clean glass slide for five seconds. The water mark and the scent marks were roughly the same size, approximately 1.2 cm × 0.3 cm. We used a different male donor's scent mark during each pairing of a female and stud male vole (n = 10 pairings per treatment). All marked slides were used in a single male-female pairing.

Immediately after the scent mark slide was prepared, we placed a female vole into the testing cage (37 × 21 × 15 cm). The female voles were injected with 0.05 mg of estradiol 60 h prior to pairing to increase the chance that the females would be receptive and mate (delBarco-Trillo & Ferkin 2004). Five minutes after the female was placed in the cage, we placed a glass slide containing a scent mark of a male donor or the control into the cage. The slide was suspended 2 cm above the substrate by a clean metal clip and hook. Five minutes after the slide was placed into the cage, we placed the subject male into the cage. We allowed the subject male to mate until sexual satiety, which is reached when no intromission occurred for 30 min (Gray & Dewsbury 1975; delBarco-Trillo & Ferkin 2004).

We recorded copulatory behavior of voles using methods similar to those detailed elsewhere (delBarco-Trillo & Ferkin 2004; Vaughn et al. 2008). Briefly, copulatory behavior of voles was recorded using a video-camcorder connected to a VCR recorder. We later scored the tapes to determine the total number of ejaculations, the latency to first ejaculation, and the mean ejaculation interval. The latency to first ejaculation was the amount of time (seconds) from the start of the trial to the first ejaculation. The mean ejaculation interval was the average amount of time (seconds) between two successive ejaculations. The methods for scoring these two variables are similar to those detailed in Vaughn et al. (2008) and delBarco-Trillo & Ferkin (2007), for examining copulatory behavior in meadow voles.

Immediately after the male reached sexual satiety, he was removed from the cage and returned to his home cage, the glass slide was discarded, and the female was removed from the cage and euthanized using an overdose of Isoflurane vapors. The female reproductive tract was removed, opened and all the ejaculate diluted in 25 ml of distilled water as detailed in delBarco-Trillo & Ferkin (2004, 2006) and Vaughn et al. (2008). The semen-water solution was gently homogenized and placed on an improved Neubauer hemocytometer to facilitate sperm counts. We used the average of four sperm counts to estimate the total number of sperm ejaculated by the male, what we considered to be his sperm allocation (delBarco-Trillo & Ferkin 2004, 2006). The experimenter was blind to the group (treatment or control) being tested.

Statistical analyses

The experimental design of this study is similar to those of delBarco-Trillo & Ferkin (2006) and Vaughn et al. (2008) in that we did not use a “within-animal” design. This was due to the difficulty of obtaining seven successful trials with the same male. Generally, not using a within-animal design may be a problem in this type of study if there is much unexplained variation among males (Pound & Gage 2004). Previous work has shown that much of the variation in sperm allocation of male voles is explained by male body size (delBarco-Trillo & Ferkin 2004, 2006). Therefore, we incorporated male body weight in the statistical analyses as a covariate. Specifically, we analyzed the data using an ANCOVA to determine if sperm allocation was affected by treatment. The main factor was treatment group (CONTROL, 1M, 24-FD, 24-RF, 72-RF, 96-RF, and 336-RF), and the covariate was male body weight. The Kolmogorov-Smirnov test was used before running the ANCOVA to test the assumption of normality. Levene's homogeneity of variance test was used to test the assumption of homoscedasticity. Statistical analyses were performed using the GLM feature in SPSS 16 for Windows. Differences were considered significant at $p < 0.05$. We used

independent GLM ANCOVAs as post hoc tests to determine significant differences between treatment groups.

Our measures of copulatory behavior were analyzed using separate one-way ANOVA's. Specifically, we determined whether males in the different treatment groups differed in the number of ejaculations, latency to first ejaculation, and mean ejaculation interval.

Results

Sperm Allocation

The sperm allocation of stud males was not affected by male weight ($F_{6, 62} = 0.697$; $p = 0.407$). However, there was an effect of treatment, with significant differences in sperm allocation among the six treatment groups and the control group ($F_{6, 62} = 3.557$; $p = 0.004$; Figure 1). We found significant differences in the sperm allocation between the stud males in the 1M condition and the stud males that were mated in the presence of the scent marks of male scent donors that were food deprived for 24 h, re-fed for 24, 72, or 96 h, or in the CONTROL condition (independent post hoc GLM ANCOVAs). First, stud males exposed to the bedding of 1 male that was not food deprived (1M condition) ejaculated more sperm than did stud males that were exposed to the no scent mark (CONTROL) condition ($F_{1,17} = 16.531$; $p = 0.001$; Figure 1). Second, it took between 96 h and 336 h of re-feeding of males that were food deprived for 24 h to induce stud males to increase their sperm allocation to levels similar to that when they were exposed to the 1M condition ($F_{1,17} = 3.248$; $p = 0.089$; Figure 1). Third, it took between 96 h and 336 h of re-feeding of males that were food deprived for 24 h to induce stud males to increase sperm allocation to levels that were significantly different than the CONTROL condition ($F_{1,17} = 4.669$; $p = 0.045$; Figure 1).

We also found that the sperm allocation of stud males exposed to donors in the 24 FD, 24 RF, 72 RF, and 96 RF treatments were statistically less than that of stud males exposed to scent donors in the 1M group ($F_{1, 17} = 11.511$, $p = 0.003$; $F_{1, 17} = 5.410$, $p = 0.033$; $F_{1,17} = 5.600$, $p = 0.030$; $F_{1,17} = 21.286$, $p < 0.0005$, respectively; Figure 1). However, the sperm allocation of stud males was similar in the CONTROL, 24 FD, 24 RF, 72 RF, and 96 RF groups ($p > 0.1$ for each comparison; Figure 1).

Copulatory Behavior

We found that different perceived risks of sperm competition did not affect aspects of the copulatory behavior of male voles. There was no significant difference among the stud males in the seven different treatment groups in their number of ejaculations ($F_{6, 63} = 1.532$, $p = 0.185$; Fig. 2a), their latency to first ejaculation ($F_{6,63} = 1.693$, $p = 0.140$; Fig. 2b), and their mean ejaculation intervals ($F_{6,63} = 1.702$, $p = 0.138$; Fig. 2c).

Discussion

We determined how long it took re-fed males that were food deprived for 24 h to be treated as a high risk of sperm competition for stud males. We discovered that 336 h of re-feeding was sufficient to induce stud males to increase their sperm allocation during ejaculation. Stud male voles did not increase their sperm allocation when they mated in the presence of scent marks of food-deprived males that were re-fed up to 96 h. This finding suggests that stud males were sensitive to changes in the nutritional status of male competitors (Engqvist & Reinhold 2005; Vaughn et al. 2008). Specifically, stud males may view a male conspecific that has been re-fed up to 96 h as being a weaker risk for sperm competition but view a male that has been re-fed for 336 h as being a strong risk for sperm competition. Vaughn et al. (2008) showed that stud male voles were able to detect differences in the scent marks of food deprived male scent donors and that they can adjust their sperm allocation

according to the nutritional state of a male conspecific. Being sensitive to the nutritional condition of competing males will allow male voles to tailor their sperm allocations prudently. Thus, the present results support and augment studies on male voles (delBarco-Trillo & Ferkin 2004, 2006), rats (Pound & Gage 2004) and fowl (Tazzyman et al. 2009) that males tailor their sperm allocation according to the risk of sperm competition (Parker & Pizzari 2010).

Our data did not support the hypothesis that food-deprived male voles that have been re-fed would quickly recover their status as being perceived as a high risk of sperm competition. Pierce and colleagues found that food-deprived female meadow voles that were re-fed for 48 h, 72 h, and 96 h were able to restore the attractiveness of their odor to males, reinstate their preference for male odors, and restore their willingness to mate, respectively (Pierce & Ferkin 2005; Pierce et al. 2005, 2007). Our results and those of Pierce and colleagues (Pierce et al. 2005) suggest that the features of the scent marks of male voles that have been altered by food deprivation and subsequent re-feeding can be detected by conspecifics. Changes in a scent donor's diet can affect its condition and may affect features of its scent mark (Ferkin et al. 1997). It is possible that this may reflect a change in the scent donor's condition brought about by re-feeding. In humans, re-feeding causes the body to switch from using carbohydrate to using fat and protein as the main source of energy, and lower basal metabolic rate by 20–25% (Veverbrants & Arky 1969; Crook 2000; Crook et al. 2001; Mehanna et al. 2008). Perhaps, re-fed male voles experience an effect from re-feeding and rival male voles can detect these changes in physiology in the former's feces and urine scent marks.

In our study, the sperm allocation of stud male meadow voles was not affected by differences in their body mass. Previous work showed that sperm allocation was affected by the body mass of male meadow voles (delBarco-Trillo & Ferkin 2004). In that study, however, the male voles we used represented a large range of body masses, from relatively small males to large males. The males used in our study were more homogenous in relation to their body masses, differing from one another by 5 g or less. Such a small variation in body mass among males could explain a lack of effect of body weight on the sperm allocation of our male voles. Consequently, differences among our male voles in their sperm allocation were not due to body mass but to differences in the stud males' perceived risk of sperm competition. This tailoring of sperm investment based on perceived risk of sperm competition is a strategy that allows male to allocate sperm prudently (Parker 1970; Dewsbury 1982; Dewsbury & Sawrey 1984; Parker & Pizzari 2010).

Stud males did not alter their copulatory behavior when they were exposed to the scent marks of male donors that were food deprived and re-fed. This finding is consistent with and augments those of previous studies, showing that stud male voles exposed to different risks of sperm competition did not alter their number of ejaculations, their latency to first ejaculation, and their mean ejaculation interval (delBarco-Trillo & Ferkin 2004, 2006, 2007; Vaughn et al. 2008). This may allow male voles to mate with multiple females (Boonstra et al. 1993; Berteaux et al. 1999) and provide each female with sufficient coital stimulation to ensure that they ovulate (Gray & Dewsbury 1975; Bakker & Baum 2000).

We found that male meadow voles do not readily overcome the effects of food deprivation. It took re-fed male voles a relatively long time, between 4 and 10 days, for rival males to perceive them as a high risk of sperm competition. Food-deprived meadow voles appear to be perceived by other males as not being a strong risk for sperm competition; these voles may have fewer mating opportunities compared to males that were not food deprived. It is not known how long food-deprived males have to be re-fed to increase their mating opportunities with females. It is possible that re-fed males enjoy a period of time when they

can mate with females without having nearby males increase their sperm allocation when mating with the same female. Such a scenario is intriguing if it allows these re-fed males an opportunity to increase their reproductive success at the expense of rival males.

Acknowledgments

This research was supported by NSF grant IOS-0444553 and NIH grant HD-049525 to M.H. Ferkin.

Literature Cited

- Bakker J, Baum MJ. Neuroendocrine regulation of GnRH release in induced ovulators. *Front Neuroendocrinol.* 2000; 21:220–262. [PubMed: 10882541]
- Batzli, GO. Nutrition. In: Tamarin, RH., editor. *Biology of New World Microtus*, Special publication no 8. American Society of Mammalogists; Lawrence, Kansas: 1985. p. 779–806.
- Bergeron J, Jodoin L. “Defining” high quality food resources of herbivores: the case for meadow voles (*Microtus pennsylvanicus*). *Oecologia.* 1987; 71:510–517.
- Bergeron J, Brunet R, Jodoin L. Is space management of female meadow voles (*Microtus pennsylvanicus*) related to nutritive quality of plants? *Oecologia.* 1990; 82:531–536.
- Berteaux D, Bety J, Rengifo E, Bergeron J. Multiple paternity in meadow voles (*Microtus pennsylvanicus*): investigating the role of the female. *Behav Ecol Sociobiol.* 1999; 45:283–291.
- Boonstra R, Xia X, Pavone L. Mating system of the meadow vole, *Microtus pennsylvanicus*. *Behav Ecol.* 1993; 4:83–89.
- Bronson, FH. *Mammalian Reproductive Biology*. University of Chicago Press; Chicago, Illinois: 1989.
- Crook MA, Halley V, Panteli JV. The importance of the refeeding syndrome. *Nutrition.* 2001; 17:632–637. [PubMed: 11448586]
- Crook MA. Lipid clearance and total parenteral nutrition: the importance of monitoring plasma lipids. *Nutrition.* 2000; 16:774–775. [PubMed: 10978859]
- delBarco-Trillo J, Ferkin MH. Male mammals respond to a risk of sperm competition conveyed by odours of conspecific males. *Nature.* 2004; 431:446–449. [PubMed: 15386011]
- delBarco-Trillo J, Ferkin MH. Male meadow voles respond differently to risk and intensity of sperm competition. *Behav Ecol.* 2006; 17:581–585.
- delBarco-Trillo J, Ferkin MH. Female meadow voles, *Microtus pennsylvanicus*, experience a reduction in copulatory behavior during postpartum estrus. *Ethology.* 2007; 113:466–473.
- Dewsbury DA. Ejaculate cost and male choice. *Am Nat.* 1982; 119:601–610.
- Dewsbury DA, Sawrey DK. Male capacity as related to sperm production, pregnancy initiation, and sperm competition in deer mice (*Peromyscus maniculatus*). *Behav Ecol Sociobiol.* 1984; 16:37–47.
- Engqvist L, Reinhold K. Pitfalls in experiments testing predictions from sperm competition theory. *J Evol Biol.* 2005; 18:116–123. [PubMed: 15669967]
- Ferkin MH, Johnston RE. Meadow voles, *Microtus pennsylvanicus*, use multiple sources of scent for sexual recognition. *Anim Behav.* 1995; 49:37–44.
- Ferkin MH, Sorokin ES, Johnston RE, Lee CJ. Attractiveness of scents varies with protein content of the diet in meadow voles. *Anim Behav.* 1997; 53:133–141.
- Ferkin MH, Li HZ, Leonard ST. Meadow voles and prairie voles differ in the percentage of conspecific marks that they over-mark. *Acta Ethol.* 2004; 7:1–7.
- Fisher HS, Rosenthal GG. Female swordtail fish use chemical cues to select well-fed males. *Anim Behav.* 2006; 72:721–725.
- Getz LL. A population study of the vole, *Microtus pennsylvanicus*. *Am Midl Nat.* 1960; 64:392–405.
- Getz, LL. Habitats. In: Tamarin, RH., editor. *Biology of New World Microtus*, Special publication no 8. American Society of Mammalogists; Lawrence, Kansas: 1985. p. 286–309.
- Gray GD, Dewsbury DA. A quantitative description of the copulatory behaviour of meadow voles (*Microtus pennsylvanicus*). *Anim Behav.* 1975; 23:261–267.

- Hobbs NJ, Aven AM, Ferkin MH. Self-grooming response of meadow voles to the odor of opposite-sex conspecifics in relation to the dietary protein content of both sexes. *Ethology*. 2008; 114:1210–1217.
- Hobbs NJ, Ferkin MH. Dietary protein content affects the responses of meadow voles, *Microtus pennsylvanicus*, to over-marks. *Acta Ethologica*. 2011a; 14:57–64.
- Hobbs NJ, Ferkin MH. Effects of protein content of the diet on scent marking and over-marking behavior in meadow voles, *Microtus pennsylvanicus*. *Behaviour*. 2011b; 148:1027–1044.
- Keller, BL. Reproductive patterns. In: Tamarin, RH., editor. *Biology of New World Microtus*, Special publication no 8. American Society of Mammalogists; Lawrence, Kansas: 1985. p. 725–778.
- Kodric-Brown A. Dietary carotenoids and male mating success in the guppy: and environmental component to female choice. *Behav Ecol Sociobiol*. 1989; 25:393–401.
- Madison DM. An integrated view of the social biology of *Microtus pennsylvanicus*. *The Biologist*. 1980; 62:20–33.
- Mehanna HM, Moledina J, Travis J. Refeeding syndrome: what it is, and how to prevent it. *Br Med J*. 2008; 336:1495–1498. [PubMed: 18583681]
- Meek LR, Lee TM. Prediction of fertility by mating latency and photoperiod in nulliparous and primiparous meadow voles (*Microtus pennsylvanicus*). *J Reprod Fert*. 1993; 97:353–357.
- Milligan SR. Induced ovulation in mammals. *Oxford Rev Reprod B*. 1982; 4:1–46.
- Parker GA. Sperm competition and its evolutionary consequences in insects. *Biol Rev*. 1970; 45:525–567.
- Parker GA, Pizzari T. Sperm competition and ejaculate economics. *Biol Rev Camb Philos Soc*. 2010; 85:897–934. [PubMed: 20560928]
- Pierce AA, Ferkin MH. Re-feeding and restoration of odor attractivity, odor preferences, and sexual receptivity in food-deprived female meadow voles. *Physiol Behav*. 2005; 84:553–561. [PubMed: 15811390]
- Pierce AA, Ferkin MH, Williams TK. Food-deprivation-induced changes in sexual behavior of meadow voles, *Microtus pennsylvanicus*. *Anim Behav*. 2005; 70:339–348.
- Pierce AA, Iwueke I, Ferkin MH. Estradiol titers, food deprivation and sexual behavior in meadow voles. *Physiol Behav*. 2007; 90:353–361. [PubMed: 17112551]
- Plath M, Heubel KU, García de León FJ, Schlupp I. Cave molly females (*Poecilia mexicana*, Poeciliidae, Teleostei) like well-fed males. *Behav Ecol Sociobiol*. 2005; 58:144–151.
- Pound N, Gage MJG. Prudent sperm allocation in Norway rats, *Rattus norvegicus*: a mammalian model of adaptive ejaculate adjustment. *Anim Behav*. 2004; 68:819–823.
- Proctor HC. Effect of food deprivation on mate searching and spermatophore production in male water mites (Acari: Unionicolidae). *Funct Ecol*. 1992; 6:661–665.
- Roberts, SC. Scent marking. In: Wolff, JO.; Sherman, PW., editors. *Rodent societies: An ecological and evolutionary perspective*. Chicago University Press; Chicago, Illinois: 2007. p. 255–267.
- Samuel MJ, Kannupandi T, Soundarapandian P. Nutritional effects on male reproductive performance in the freshwater prawn *Macrobrachium malcolmsonii* (H. Milne Edwards). *Aquaculture*. 1999; 172:327–333.
- Tamarin, RH., editor. *Biology of New World Microtus* Special publication no 8. American Society of Mammalogists; Lawrence, Kansas: 1985.
- Tazzyman SJ, Pizzari T, Seymour RM, Pomiankowski A. The evolution of continuous variation in ejaculate expenditure strategy. *Am Nat*. 2009; 174:E71–E82. [PubMed: 19627229]
- Vaughn AA, delBarco-Trillo J, Ferkin MH. Sperm investment in male meadow voles is affected by the condition of the nearby male conspecifics. *Behav Ecol*. 2008; 19:1159–1164. [PubMed: 19529815]
- Veverbrants E, Arky RA. Effects of fasting and refeeding. I Studies on sodium, potassium and water excretion on a constant electrolyte and fluid intake. *J Clin Endocrinol Metab*. 1969; 29:55–62. [PubMed: 5762322]

Fig. 1.

The mean $\pm$ SEM sperm allocation of stud males exposed to no scent mark (CONTROL), the scent mark of an unrelated, unfamiliar male conspecific (1M), the scent mark of a 24-hour food-deprived male (24-FD), and the scent mark of a re-fed male: 24-RF, 72-RF, 96-RF, and 336-RF. Asterisks indicate significant differences between groups *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Fig. 2.

The mean $\pm$ SEM (a) number of ejaculations, (b) latency to first ejaculation (seconds), and (c) mean interval (seconds) between ejaculations, by males exposed to no scent mark (CONTROL), the scent mark of an unrelated, unfamiliar male conspecific (1M), the scent mark of a 24-hour food-deprived male (24-FD), and the scent mark of a re-fed male: 24-RF, 72-RF, 96-RF, and 336-RF. There were no significant differences between groups.