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Copulation and ejaculation in male rats under sexual satiety and the Coolidge effect

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ABSTRACT

Sexually satiated males cease copulating after several ejaculations with the same female; and the presence of an unknown receptive female renews copulation including ejaculation, a process named the Coolidge effect. It is believed that the Coolidge effect has the aim to impregnate another female, although it is known that the sperm count gradually decreases after consecutive ejaculations. The main goal was to investigate if sexually satiated males during the Coolidge effect can reestablish seminal expulsion associated to the ejaculation behavior and/or penile erection associated to the intromission behavior. The results show that during the Coolidge effect, most of the sexually satiated males showed the motor ejaculatory behavior, however, no sperm in the uterine horns or seminal plug in the vagina were detected. Such lack of sperm was not related with the number of ejaculations required to achieve sexual satiety nor with the number of intromissions needed for ejaculating (experiment 1: 2.4.1.). After the behavioral ejaculation, during the Coolidge effect, there was a 44% decrease in sperm count in the epididymal caudae (experiment 1: 2.4.2.). Males that mated for 8 behavioral ejaculations (close to sexual satiety) deposited tiny seminal plugs but no sperm in the female reproductive tract (experiment 1: 2.4.3.). Interestingly, sexually satiated and non-satiated-animals displayed similar number of intromissions and spent a similar time in dislodging the seminal plug from the vagina deposited by other males (experiment 2). These results suggest that sexually satiated males during the Coolidge effect have the capacity for penile erection and vaginal insertion, because they are able to dislodge seminal plugs; but are unable to expel seminal fluid, because neither form seminal plugs nor deposit sperm in the female genital tract.

1. Introduction

The Coolidge effect is a behavioral paradigm characterized by the reestablishment of male sexual activity after satiety when an unknown estrous female is presented to the previously satiated male [1]. The Coolidge effect has been studied using various paradigms; including multi-female tests, changes of the female before sexual satiety and changing conditions [2]. This effect has been studied in different mammals including rats [3], cats [4], sheep [5], hamsters [6], and montane voles [7], among others. In recent years, the Coolidge effect has also been demonstrated in burying beetles. When these beetles are allowed free social encounters, males copulate but avoid mating repeatedly with the same female, recognizing her by cuticular odors [8]. Most studies propose that the biological significance of the Coolidge effect is to promote higher reproductive possibilities by preventing males from continued copulation with the same female and facilitating mating with other females [9].

Because sperm production has nontrivial costs, it has been proposed that copulation with different females during the Coolidge effect is intended to increase the genetic diversity of the males' offspring [10]. However, successive ejaculations produce two parallel actions: a reduction in sperm count analyzed in the female reproductive tract [11,12] and in the male epididymis and vas deferens [13] — accompanied by a diminished seminal plug weight and size [11,12] — and an inhibition of sexual behavior. In rats, for example, males ejaculate 8–12 times with the same sexually receptive female [14]; during this ~2.5 h of constant copulation [15] the postejaculatory intervals increase until the male ceases mating and can be categorized as sexually satiated [16,17]. As aforementioned, the introduction of a new sexually receptive female shortly after sexual satiety induces the Coolidge effect: a renewed bout of mating for another ~2.5 h [14,18,19].

Therefore, we asked if this renewed behavioral motor pattern of ejaculation after sexual satiety — during the Coolidge effect — is accompanied by an absence of ejaculate in the female reproductive tract due to a reduction in sperm count in the epididymis cauda (experiment 1). Previous studies show a drastic reduction in sperm concentration in the whole female reproductive tract after seven ejaculations each with different female [11,12]. In this report we studied if a sperm reduction is

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observed after sexual satiety during the Coolidge effect and after the eighth behavioral ejaculation, when animals are close to sexual satiety and have copulated with the same female repeatedly (experiment 1). Secondly, we compared if sexually satiated males during the Coolidge effect differ from non-sexually satiated animals in their ability to remove the seminal plug deposited by another male (experiment 2).

2. Material and methods

2.1. Animals

Male and female Wistar rats (around 300 and 200 g body weight, respectively) were obtained from the *vivarium* of the Universidad Autónoma de Tlaxcala. Males were sexually experienced, whereas ovariectomized females were used in copulatory — and seminal parameters — tests. All animals were kept under standard conditions, with controlled temperature (21 ± 2 °C) and relative humidity 30 to 45%, under a 12:12 light–dark cycle (lights on at 0800 h), with free access to rodent food pellets (Purina Chow, México) and water. All procedures were done in accordance with the guidelines of the Laws and Codes of México (Seventh Title of the Regulations of the General Law of Health Regarding Health Research) and also followed the guidelines of the NIH for the use of Animals [20]. All the procedures were approved by the Institutional Animal Care and Use Committee.

2.2. Copulatory tests

Sexually experienced males and ovariectomized virgin females were used. Females were brought into behavioral estrus by the sequential treatment with 10 μg of estradiol benzoate (Sigma-Aldrich, St. Louis, MO, USA,) and 2 mg of progesterone (Sigma-Aldrich) administered by subcutaneous injection 44 h and 4 h before the copulatory encounters, respectively. All the steroids were dissolved in olive oil and administered subcutaneously in the neck region. The rats were allowed to mate in Plexiglas cylinders (50 cm diameter and 50 cm height) with wood shavings covering the floor.

The copulatory variables we recorded were: (a) the mount and intromission latencies, defined as the time in seconds from the start of a test (when the female was introduced into the copulatory arena) to the first mount or intromission, (b) the number of mounts (number of mounts with pelvic thrusting in an ejaculatory series), (c) the number of intromissions (number of mounts with vaginal penetration in an ejaculatory series), and (d) the number of ejaculations until sexual satiety [3]. Males were considered sexually satiated when they were totally inactive for 30 min after the last ejaculation. All sexual behavior tests were done in the second third of the dark phase under dim red light illumination.

2.3. Evaluation of uterine fluid content and seminal plugs

Once the ejaculatory behavioral motor pattern was observed in the first ejaculatory series and after the first ejaculation produced by the Coolidge effect or in the eighth copulatory series, the female was immediately transferred from the Plexiglas cylinder to an empty cage where she was left for 5 min before being anesthetized with sodium pentobarbital (26 mg/kg, i.p.; Pfizer, México). After a medial abdominal incision, the uterine horns were tied proximally and distally using 000 silk threads, removed from the abdominal cavity, and immersed in a Petri dish containing physiological saline solution at 37 °C. This procedure allows the elimination of blood, fat tissue and external uterine vessels, besides serving to maintain the uterine fluid under a controlled temperature for adequate spermatozoa survival. The uterine fluid content of both uterine horns was placed into a 1.5 mL micro-centrifuge tube and maintained in a thermo bath (37 °C). The uterine fluid sample color was distinguished as off-white or transparent. The seminal plug was removed from the vagina by separating the pubic symphysis and making an incision in the vaginal wall. The seminal plug was detached from the lateral and ventral vaginal walls plus the cervix.

The variables evaluated in the uterine fluid content were (a) viscosity, measured by the length of a thread formed after introduction and withdrawal of a tip of a transfer pipette into the semen, expressed in millimeters; (b) sperm count, the number of spermatozoa expressed in millions and (c) sperm motility, the number of spermatozoa that moved progressively (with advanced movements), moved *in situ* (circular or local movements) or that were immobile, expressed as percentage. Cytological elements, including the number of single heads, single flagellum and complete spermatozoa were given as percentages relative to their total number. The seminal plugs obtained from normal male rats have a cup shape at the proximal end attached to the cervix [21] and acquire the shape of the vaginal tract, being more wide at the proximal end and tiny at the distal end. That is, the body of the plug has a cone-like form. For that reason it is possible to use an arithmetic formula to measure the cone volume: $(\text{length})(\pi)(r^2)/3$, calculating the radio as half the width of the plug's equatorial region. This is a quantitative approach to obtain the plug volume [22,23]. The size (length and width in mm), weight (in mg) and volume (in cubic millimeters) of the seminal plug were determined; all measurements were made using a vernier. After collecting the semen and the seminal plug, the females were euthanized using an additional injection of sodium pentobarbital (50 mg/kg, i.p.; Pfizer, México) [22,23].

2.4. Experiment 1

2.4.1. Comparison of the copulatory, uterine fluid content and seminal plug variables between the first ejaculatory series and after sexual satiety, during the Coolidge effect

We compared the behavioral copulatory characteristics, the uterine fluid content and seminal plug features between the first ejaculatory series and that observed during the Coolidge effect; that is, after the animals ceased copulating with a given female and renewed sexual activity with a new — unknown for the male — sexually active female. This protocol was used to determine if males induced to mate by the Coolidge effect were able to show seminal expulsion. Eighteen males were used, and each of them copulated with three different females. The first female was used to analyze the ejaculate variables of the first ejaculatory series. She was sacrificed after the male displayed the first behavioral motor pattern of ejaculation. A second female was introduced and allowed to copulate from the second ejaculatory series onwards until the male attained sexual satiety. Finally, a third female was used to produce the Coolidge effect. After the male showed the behavioral pattern of an ejaculation, she was removed and euthanized to analyze the ejaculate. The copulatory parameters of the first ejaculatory series and those of the Coolidge effect were compared.

2.4.2. Comparison of sperm count in the epididymis caudae between the first ejaculatory series and after sexual satiety, during the Coolidge effect

Twelve males were used, six of them copulated until sexual satiety and the others were allowed only one ejaculatory series. Immediately after males reached their respective behavioral endpoint, a combination of ketamine and xylazine (90 mg/kg and 15 mg/kg, i.p.; Virbac S.A. and Bayer, México) was administered, which produced rapid anesthesia and muscle relaxation allowing fast access to the epididymis. Both epididymides were exposed by a ventral incision on the scrotal raphe. Two ligatures were done to obtain the epididymis caudae, one in the distal portion of the epididymal body and another in the proximal portion of the vas deferens. The caudal portion of the epididymis was immersed in a Petri dish containing saline solution at 37 °C and the blood, fat tissue and debris were carefully removed. After the epididymis cauda was weighed and measured (length, width), it was placed over a slide with an excavation containing 60