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Estrous cycle staging before mating led to increased efficiency in the production of pseudopregnant recipients without negatively affecting embryo transfer in mice

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The goal was to increase pseudopregnant mice production by estrous cycle staging by visual examination before pairing and to determine the effect of such pseudopregnant recipients on embryo transfer. To compare methods of estrous cycle staging over 14 days, groups consisted of 10 females in proestrus-estrus and 10 vasectomized males; group 1: only daily visual observation; group 2: daily visual observation and cytological examination on day 1; group 3: daily visual observation and daily cytological examination. The average time to first vaginal plug was 1.8 days in group 1, 2.7 days in group 2, and 3.2 days in group 3, whereas the average time between consecutive vaginal plugs was 9.2 days (group 1), 10 days (group 2), and 9.25 days (group 3). The average time between consecutive estrous cycles was 9.7 days (group 1), 11.8 days (group 2), and 9.4 days (group 3). The congruence between visual and cytological examination in determining proestrus-estrus in group 2 was 100% and that for the four stages in group 3 was 79% with a range of 44% to 100%. From 162 plug-positive females originally selected in proestrus-estrus, 49%, 30%, 19%, and 2% were plug-positive on Day 1, Day 2, Day 3, and Day 4, respectively, showing that pseudopregnant mice production was significantly increased on the first 2 days. From 192 plug-positive females originally selected randomly, these values were 31%, 21%, 35%, 10%, and 3% on d1, d2, d3, d4, and d5, respectively. No significant differences were observed between groups with respect to embryo transfers with fresh or cryopreserved embryos although the number of pups born per litter was higher in group A with fresh (7.57 vs. 6.39) and cryopreserved-thawed embryos (5.0 vs. 4.38). Furthermore, the sex ratio and the genotype of the pups were not significantly affected.

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1. Introduction

Besides animal husbandry itself, many contemporary mouse facilities usually perform work in the field of genetic engineering and assisted reproductive technologies. The latter tasks are coupled with the need for embryo transfers (ETs) and, as such, for pseudopregnant recipients. Therefore, these different tasks represent competing interests, and often, the number of pseudopregnant recipients can become limiting. To produce ET recipients, vasectomized males are mated with females at an appropriate age. These females are usually selected randomly without determining the stage of the estrous cycle. The mating success is influenced by many factors including male performance and, moreover, by taking advantage of the Whitten effect, whereby females are usually mated within 3 days after placing them with the males [1].

The estrous cycle of the mouse consists of proestrus, estrus, metestrus and diestrus and usually lasts 4 to 6 days [2–4]. However, its length is highly variable due to genetic and environmental factors. Studying 1000 cycles in unmated albino mice, the lengths of the estrous cycle were

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for 2 days, 2%; 3 days, 3%; 4 days, 16%; 5 days, 29%; 6 days, 22%; 7 days, 12%; 8 days, 6%; 9 days, 3%; and 10 to 28 days, 8% [5]. The combined proestrus-estrus stages lasted an average of 2.4 days, and the combined metestrus and diestrus stages averaged 3.7 days [5]. This means that approximately 45% of the females have a 4 to 5-day cycle. Because the estrus stage usually lasts up to 14 hours, this means that at any time point with a 4 to 5-day estrous cycle, approximately 12% to 15% of randomly cycling females should be in estrus. To obtain a certain number of pseudopregnant females on a particular day, approximately 6 to 8 times as much randomly cycling females need to be paired. In contrast, selection of mice in proestrus-estrus means that these mice should be more receptive to males and, thus, to mating [6], leading to a higher efficiency in the production of pseudopregnant females.

The stages of the estrous cycle can be determined by measuring electrical impedance [7], biochemical analysis of urine [8], cytological examination of vaginal smears [2,6,8–11], and visual examination of the vagina [6,9]. In the literature, there are more reports on the latter two methods.

The cells lining the vagina of the female mice respond to the different levels of hormones circulating and therefore are related directly to the state of the reproductive organs and the potential for ovulation and mating. Cytological examination involves the identification of the different cell types and their proportions in samples collected from the vagina by scraping, swabbing, or lavages. These samples are then stained and examined microscopically. Cytological examination was the routine method for determining the estrous stage in many different species [2,10,12–16]. Since changes in the appearance of the mouse vagina during the estrous cycle were first reported by Allen [2], many workers have adopted the technique of visual examination [6,9]. The latter depends on recognizing changes in the appearance of the vagina during the estrous cycle including swelling, color and moistness of the tissues, the size of the vaginal opening, and the presence of white cellular deposits in the vagina.

In mice, the success at obtaining and carrying a pregnancy to term after ET depends on many factors including the genetic background of the donor and recipient strains used [17–19], age [20] and the weight of the recipients, stage-synchrony of recipients [20,21], hormone status of recipients [22], embryonic stage [21,23], embryo quality [24], number of embryos [21,23,25,26], unilateral or bilateral transfer [21,27], the experience of the person performing the transfer, and husbandry conditions including noise level [28,29].

Recently, Byers et al. [6] reported that selection of mice in the proestrus and estrus stages by the visual method before pairing can be used for the production of pseudopregnant recipients. However, these workers made no comparisons between selection of female mice by estrous cycle staging and random selection with respect to the efficiency of pseudopregnant female production. Furthermore, they did not present any work on ET with the pseudopregnant recipients produced by estrous cycle staging before pairing.

The present goal was to first determine the different stages of the estrous cycle by visual examination of the vagina and cytological examination of vaginal smears and to assess the congruence of these two methods as visual examination can be subjective, varying according to the investigator. Second, females were selected in the proestrus-estrus stage or randomly before pairing for the production of 0.5-day-old pseudopregnant recipients to determine which method of selection of females leads to a higher number of pseudopregnant recipients. Finally, ETs with pseudopregnant recipients produced by both methods of selection were also performed to determine whether the method of selection of the females affects reproductive parameters.

2. Materials and methods

2.1. Mice and husbandry

Outbred Crl:CD1 (ICR) (CD-1) mice were used in the present experiments for the production of vasectomized males and pseudopregnant recipients. This colony was originally introduced via ET and bred in the full barrier unit of the animal facility at the Center for Molecular Medicine, University of Cologne. Breeding colonies were kept in individually ventilated cages (IVCs; Tecniplast, Italy) at a temperature of 20 °C to 24 °C, humidity of 50% to 60%, 60 air exchanges per hour in the cages, and a 12/12-hour light/dark cycle with the lights on at 5:30 AM. The maximum caging density was five mice from the same litter and sex starting from weaning. As bedding, spruce wood shavings (Lignocel FS-14; J. Rettenmaier und Soehne GmbH, Rosenberg, Germany) were provided. Mice were fed a standardized mouse diet (1314, Altromin, Germany) and provided drinking water *ad libitum*. All materials, including IVCs, lids, feeders, bottles, bedding, and water were autoclaved before use. Sentinel mice were negative for at least all Federation of laboratory animal science associations (FELASA)-relevant murine infectious agents [30] as diagnosed by our health monitoring laboratory, mfd Diagnostics GmbH, Wendelsheim, Germany. All females intended to be used as recipients were housed in the same room. Before selection and pairing, females were allowed a 1-week acclimatization period in the room where the vasectomized males were kept although conditions in each room were as described previously.

2.2. Visual examination of the estrous stages

Visual examination of the estrous stage was performed based on the criteria described by Champlin et al. [9] and as shown in Figure 1 with our photographs (visual). In a sterile laminar hood, by holding the tail, females were restrained on the grid of the cage with their front paws. The rear end of the mouse was lifted slightly above the grid toward the investigator. The vagina was observed under normal lighting (30 Watt) in the sterile hood, and additional lighting was provided by 36-Watt fluorescent ceiling lights in the mouse rooms. The appearance of the vagina was photographed with a camera (Panasonic model DMC-T24).

2.3. Cytological examination of the estrous stages

Cytological examination of the estrous stage was performed as described by McLean et al. [31]. Females were restrained on the grid of the cage as described previously. A 100-μL drop of sterile water was expelled with a 100-μL

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