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Pregnancy in a Breastfeeding Mouse without Mating

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Abstract

In this report, a rare case of pregnancy in a mouse without mating and during breastfeeding is presented. Out-bred NMRI mice were prepared for mating. Two female mice without superovulation were caged with one male. The next morning, vaginal plaque was not detected; therefore, females were kept in the cage with the same male for 4 more days. One of the females delivered 4 offspring: one female and three males. This female conceived again without mating, 21 days after the first delivery and during the breastfeeding period and this time delivered 16 offspring: 13 males and 3 females. The more likely explanation for this rare, second parturition is embryonic diapause.

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Introduction

Infant nourishment by breastfeeding results in temporary postpartum infertility (1, 2). This period is accompanied by prolactin enhancement and a decrease in gonadotropin levels, leading to amenorrhea and anovulation (3-5). In fact, because of this lactation induced infertility, breastfeeding forms the basis of a family planning method known as the lactational amenorrhea method. Human plasma concentrations of prolactin and estradiol in the 38th week after pregnancy directly correlate with the duration of lactational amenorrhea (6). Parthenogenesis or parturition without mating is a form of unisexual reproduction in which development and growth of embryos occur without fertilization (7, 8). There are approximately 80 known unisexual species of reptiles, fishes and amphibians, while parthenogenesis is very rare in mammals (9, 10). In the present study, we report a mouse pregnancy immediately after parturition, without mating, and survey possible reasons for this infrequent phenomenon.

Case report

NMRI out-bred mice were purchased from Tehran Pasture Institute and were kept in 12h/day light cycles at ambient temperature (23±3°C) for two weeks. Animals had free access to water and commercial food. Two female mice without superovulation were mated with one male. The next morning, vaginal plaque was not observed. Therefore, the two female mice were kept in the cage with the male for an additional four days. Upon observation of vaginal plaque, they were placed in separate cages. After 21 days, one of the mated females gave birth to 4 offspring: 3 males and 1 female. Twenty-one days after this first parturition, while the newborn pups were being breastfed, and without mating, this female gave birth to 16 offspring: 13 males and 3 females.

Discussion

In this report we describe a very rare phenomenon; the occurrence of a second conception while newborns from the first parturition were still being breastfed, and without mating. In the remainder of this work we will examine the various possibilities and attempt to determine the most likely explanation for the presented case.

In most mammalian species, fertility is suppressed by lactational amenorrhea (1, 2). Afferent neural impulses from nipple suckling pass through the spinal cord to the hypothalamus, where they cause a local release of beta endorphin, resulting in suppression of GnRH release and inhibition of pituitary FSH and LH secretion, ovulation and menstruation. Lactational amenorrhea also inhibits dopamine production (prolactin inhibiting factor), resulting in increased pituitary prolactin secretion. Increases in suckling frequency are associated with increased beta endorphin production that results in longer lactational amenorrhea duration (11-13).

The contraceptive effect of breastfeeding in women is ≥ 98% in the first 6 postpartum months (11). Breastfeeding delays the resumption of normal ovarian cycles via a disturbance in pulsatile GnRH (gonadotrophin releasing hormones) release from the hypothalamus, and also LH secretion from the hypophysis, resulting in ovulatory quiescence during lactation (14).

In addition, during lactation, prolactin may reduce Leptin blood concentrations. Leptin, a protein hormone, may induce GnRH secretion from the hypothalamus. Therefore, a secondary effect of prolactin is reduction of gonadotropin release from the pituitary gland (15). In spite of this, Tay et al. suggest that there may be no precise link between prolactin release and the duration of lactational infertility in breastfeeding women (16).

Estrogen also plays an important role in beta endorphin release. During lactation, the negative feedback effect of estrogen (long loop) on the hypothalamus-hypophysis axis increases, reducing GnRH release from the hypothalamus (6). Furthermore, although plasma FSH concentrations are sufficient to stimulate follicle development during lactation, an inadequate pulsatile LH signal results in reduced estradiol production by these follicles. When follicle development proceeds and estradiol secretion increases, nipple suckling prevents the generation of a normal preovulatory LH surge and consequently follicles do not rupture and ovulation does not take place (17, 18).

The other possible explanation for the pregnancy reported in this study could be long-term survival and preservation of sperm in the female's reproductive system, or embryonic

diapause. In theory, if adequate nutrients are available, sperm can survive for a long time (19). In fact, although there are no reports of long-term sperm preservation in mice, other reports indicate that sperm can be maintained for an extended period of time in animals such as birds. Female birds are able to maintain viable sperm for several weeks, but the duration of sperm preservation in male birds is short (20, 21). Fertilized eggs in hens are generated several weeks after the insemination. The main preservation sites for sperm in hens are specialized sperm storage tubules (SSST) in the uterovaginal junction, slightly distal to the uterus. In most mammalians, sperms are stored in a quiescent status (22, 23). Generally, acidic pH decreases motility, while alkaline pH has the opposite effect. Therefore, pH is an important factor for sperm storage in a non-motile state. The existence of carbonic anhydrase (CA) connected to SSST membranes and infundibular storage of sperm may provide the acidic conditions necessary for diminution of sperm motility (24).

In hibernating insectivorous bats from temperate latitudes, mating takes place before hibernation (late summer), but ovulation, fertilization and implantation are delayed until the spring (25). It is possible that this delay is provided by either long-term sperm storage, in conjunction with delayed ovulation, fertilization and implantation of early fetus, or embryonic diapause (26). According to Kitchener, spermatozoids are stored in the uterine gland and the uterine part of the oviducts (27). In fact, the female reproductive system of bats is able to store fertile spermatozoids for 33 to 198 days (25, 28). However, it is also possible that long-term sperm storage *in vitro* can affect the development of the resulting embryo due to defective DNA and protein synthesis (29). Salisbury et al., for the first time in 1961, showed that both the quantity (sperm number) and quality (sperm fertility) of sperm are important (30). In oxygenated environments, the production of different types of reactive oxygen species (31), such as O_2, H_2O_2 and OH free radicals is unavoidable (19). These free radicals may damage or destroy nuclear chromatin. Peroxide is the most pernicious (destructive) pro-oxidant, and is produced by membrane-bound aromatic amino acid oxidases (AAAO) from dead sperm. Interestingly, this enzyme is inactive in live sperm (32). Seminal plasma, which is an ideal environment for sperm motility and fertility after ejaculation, has antibacterial properties and protects sperm from peroxide damage. However, cow seminal plasma has an automatic destructive effect on sperm (19, 33).

Kono reported that genomic imprinting acts as a barrier to parthenogenesis in mammals. Also parthenogenetic embryos of mouse die by day 10 of gestation (34, 35). Therefore, in spite of parthenogenesis occurance in some of reptiles, amphibians and fishes, it is an unusual phenomenon in mammals (10).

Embryonic diapause (delayed development) is also seen during developmental arrest in the blastocyst stage of the embryonic period in mammals (36). In fact, development of the embryo is temporarily stopped for various periods, due to suppression of cell proliferation. Nonetheless, the precise mechanisms of embryonic diapause remain unknown, and embryonic diapause must be considered as another possible explanation for the mouse pregnancy presented here. Embryonic diapause has been observed in *mus musculus* (common house mouse) by Spindler et al (37).

In some animal species, such as brown rabbits, spontaneous ovulation, pregnancy and subsequent embryo development are able to occur while another fetus is still

present in the uterus (31). In these animals, ovulation and superfoetation are observed at levels up to 60 percent. In late pregnancy, increased mating frequency can result in increased LH surge. This rare phenomenon, called superfoetation, has been reported in humans as well (38).

Conclusion

Because the number of offspring in the first parturition of our case was low (three pups), there is a possibility that nipple suckling was not sufficient to induce a suppressive effect on gonadotropin release. In addition, the majority of the pups from the second parturition were male and given that the longevity of Y versus X sperm is lower, the chance of long-term sperm survival for a subsequent round of fertilization is low. Therefore, the more likely explanation for this rare, second parturition is embryonic diapause.

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