Review Report

Embryonic diapause: a unique stage of development

Theresa Beachler
Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA

Abstract

Embryonic diapause is a unique stage of development that occurs in some mammalian species. Diapause serves as an evolutionary reproductive strategy to maximize the survivability of dam and offspring. Although individual mechanisms of control vary among species, the overarching physiology of diapause extends the length of pregnancy to delay parturition until either seasonal, environmental, nutritional, or internal physiologic constraints are optimal for parturition and rearing of young. This review discusses concepts and highlights control mechanisms unique to rodents, mink, and tammar wallabies.

Keywords: Embryonic diapause, development, blastocyst, pregnancy

Introduction

Embryonic diapause, also known as delayed implantation, is a temporary state of arrested development that occurs in some mammals at the blastocyst stage. Embryonic diapause is an evolutionary strategy to delay parturition to ensure the optimal seasonal, nutritional, environmental, and physiologic conditions occur for both survivability and the rearing of offspring. Diapause was first noted in roe deer (*Capreolus capreolus*) in the mid-19th century.[1] Subsequently, it was observed in over 130 species across various orders ranging from rodents, marsupials, numerous carnivores, including minks, weasels, skunks, bears, seals, armadillos, bats, and some species of deer.[2,3] The defining characteristic of diapause is the dramatic reduction or cessation of cellular activity in the embryo at the G0/G1 stage.[3] In some species (e.g. mice and marsupials) cellular activity stops in its entirety with cessation of all cell divisions, transcription, and translation, whereas in others, such as the roe deer and some carnivores (mink, weasels, and bears), growth and activity remains but at an incredibly slow rate.[3,4] In roe deer, for example, ~ 10% of cells proliferate where the embryo expands from 300 to 20,000 cells over a period of 4 months, with the majority of growth occurring within the trophectoderm compared to the inner cell mass.[4] Maintenance or length of delayed development may range from a brief halt lasting for a few days, as observed in mice and rats (4-10 days), to much longer extended period of ~ 10 months in the badger and up to 11 months in the tammar wallaby.[2]

Types of diapause

Diapause is classified into 2 categories, either facultative or obligate diapause, with most species, excluding the tammar wallaby, experiencing 1 or the other.[1-3,5] Facultative diapause occurs in response to a limiting internal physiological factor.[2,3,5] Facultative diapause is also sometimes referred to as lactational diapause and is best understood in mice and some marsupials, where development is delayed in the presence of suckling young until weaning occurs.[4] Obligate diapause or seasonal diapause has been best studied in carnivores, where delayed development occurs in every pregnancy under a seasonal photoperiod control.[5] For example, in many carnivores residing in the Northern Hemisphere, such as the mink or skunk, and in bears, development is delayed until decreased melatonin is produced from the pineal gland in response to the onset of long days to support the birth and rearing of young during the most favorable environmental and nutritional conditions.[2,5] Therefore, the hormone prolactin is a key player in both varieties of diapause with marked interspecies variability.

Species-specific examples of lactational and seasonal control

Mice

Rodents such as rats and mice experience the first postpartum estrus within 24 hours of parturition.[6,7] In mice

CONTACT Theresa Beachler **beachler@iastate.edu**

Citation line: Clinical Theriogenology 2024, 16, 9400, http://dx.doi.org/10.58292/CT.v16.9400

(*Mus muscularis*) that were successfully mated, lactation in the presence of suckling young results in elevated concentrations of prolactin.[6-8] High concentrations of prolactin prevent the estrogen surge that occurs at 3.5 days postovulation, initiating a facultative or lactational diapause for a period of 1 day to several weeks.[5,8] Larger litter sizes additionally have been associated with longer periods of delayed development.[7] Following weaning and the removal of the inhibitory effects of prolactin, embryos are reactivated by the estrogen surge and subsequent elevations in progesterone concentrations that prepare the endometrium for implantation.[5] This has been supported experimentally by antagonist/dopamine agonist (bromocriptine) treatment, which terminated diapause and induced reactivation and implantation.[9] Embryo transfer in ovariectomized female rats that were treated with progesterone prior to the natural estrogen surge, entered into an arrested state with reactivation occurring after estradiol treatment.[10]

Mink

American mink (*Neovison vison)* is 1 of the best studied obligate or seasonal diapause family members. In American mink residing in the Northern Hemisphere, embryo development is delayed until decreased melatonin production occurs from the pineal gland in response to the onset of long days or > 12 hours of daylight after the spring equinox.[11-13] Mink breed during spring, with matings occurring between February and April. Subsequently, prolactin concentrations remain low in response to high nocturnal melatonin production (< 12 hours of daylight). Regardless of the time of mating and ovulation, lengthening photoperiod and concurrent reduction in melatonin increases circulating prolactin concentrations, resulting in ovarian (corpora lutea) produced progesterone increase.[11,12] On the contrary in rodents, experimental treatment with the dopamine agonist bromocriptine extended diapause, whereas treatment with the dopamine antagonist pimozide terminated diapause.[14,15] Therefore, prolactin is directly luteotrophic, ending diapause and resuming embryonic development in mink.

Wallabies

Tammar wallabies (*Macropus eugenii*) are unique as they may undergo both a facultative and obligate diapause in response to elevated concentrations of prolactin for a total period lasting up to 11 months.[5] In the Southern Hemisphere, tammar wallabies typically give birth to a single joey in late January to early February and enter a postpartum estrus shortly after, with mating occurring as early as 1 hour after birth.[16,17] In successfully bred females, high concentrations of prolactin from the suckling young contribute to the initiation of lactational diapause. If the joey is lost during the breeding season (January through May), embryonic development resumes in the absence of high prolactin concentrations.[18] After the winter solstice (June), only the seasonal photoperiod is sufficient to cause reactivation, with no response if joey is lost after this period. After the subsequent summer solstice (December), prolactin concentrations decrease in response to decreased photoperiod and increase in melatonin, allowing for blastocyst reactivation.[18,19] Tammar wallabies, therefore, are unique species as they may display either a single or a combination of a facultative or obligate diapause depending on the time of year and if a suckling joey is present during periods of pregnancy.

Additional mechanisms for reactivation or cessation of diapause

At the blastocyst stage, embryos of species that undergo diapause have not yet initiated implantation and remain within the uterine lumen either within (marsupials and carnivores) or hatched from the zona pellucida (rodents, roe deer, and armadillos). In addition to varied hormonal changes as discussed above under lactational or seasonal control, reactivation or the cessation of the period of diapause appears to also be mediated by a combination of cytokines, growth factors, and transcriptional factors produced from the uterine endometrium within uterine secretions and the embryo itself, all of which is not completely understood.[2] At the arrested blastocyst stage, embryos have yet to initiate implantation and may not begin the process until multiple days after reactivation. Factors identified with altered patterns of expression during reactivation are conserved across many species include epidermal growth factors (EGF and HB-EGF), leukemia inhibitory factor (LIF), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), platelet-activating factor (PAF), transforming growth factor (TGF-β), interleukin 1β (IL1β), bone morphogenic protein-2 (BMP2), and prostaglandin synthetase PTGS2 (COX2).[1,5,20-22] In mice, leukemia inhibitory factor (LIF) has a critical molecule in reactivation and the onset of implantation as its expression is substantially upregulated within the endometrial glands of pregnant mice on days 4 and 5 of pregnancy.[21,22] Female mice lacking functional LIF genes produced viable embryos that failed to implant at the blastocyst stage in vivo but underwent appropriate development when transferred to wild-type recipients.[22] Similarly, epidermal growth factor (EGF) has been critical for implantation in mice and rats with knockout receptor dams failing all embryonic development beyond the blastocyst stage with degradation of the inner cell mass.[23] Additionally, signaling molecules or transcription factors encoded by the wingless family (WNT) and muscle segment homeobox genes (MSX1 and MSX2) were involved in activation and implantation in mice, mink, and wallabies.[1,2,24-27] WNT signaling may also contribute to the altered spacing of embryos which in some species occurs simultaneously with embryo reactivation.[24] Muscle segment homeobox genes (MSX1 and MSX2) are further mediated by WNT5a signaling with marked downregulation of these genes and their products occurring at embryo reactivation and implantation in mice, mink, and wallabies.[24,25]

Conclusion

Mammalian embryonic diapause is a phenomenon defined by a temporary arrest in blastocyst development as a reproductive strategy to promote both the survival of the dam and neonate until either environmental or physiologic factors are favorable. Although a whole host of variability is demonstrated, many of the hormonal and signaling factors are highly conserved.

Conflict of interest

None.

References

1. Fenelon JC, Renfree MB: The history of the discovery of embryonic diapause in mammals. Biol Reprod 2018;99:242-251. doi: 10.1093/biolre/ioy112

2. Renfree MB, Shaw G: Diapause. Annu Rev Physiol 2000;62:353-375. doi: 10.1146/annurev.physiol.62.1.353
3. Lopes FL, Desmarais JA, Murphy BD: Embryonic diapause and its regulation. Reproduction 2004;128:669-678. doi: 10.1530/rep.1.00444
4. Rüegg AB, Bernal S, Moser FN, et al: Trophectoderm and embryoblast proliferate at slow pace in the course of embryonic diapause in the roe deer (*Capreolus capreolus*). Biosci Proc 2020;10:181-196. doi: 10.1530/biosciprocs.10.013
5. Renfree MB, Fenelon JC: The enigma of embryonic diapause. Development 2017;144:3199-3210. doi: 10.1242/dev.148213
6. Long JA, Evans HM: The oestrous cycle in the rat and its associated phenomena. Mem Univ Calif 1922;6:1-148.
7. Weichert CK: The experimental shortening of delayed pregnancy in the albino rat. Anat Rec 1940;77:31-47. doi: 10.1002/ar.1090770105
8. Mantalenakis SJ, Ketchel MM: Frequency and extent of delayed implantation in lactating rats and mice. J Reprod Fertil 1966;12:391-394. doi: 10.1530/jrf.0.0120391
9. Flint AP, Renfree MB: Bromocriptine-induced implantation during lactation in the rat. J Reprod Fertil 1981;62:181-183. doi: 10.1530/jrf.0.0620181
10. Yoshinaga K, Adams CE: Delayed implantation in the spayed, progesterone treated adult mouse. J Reprod Fertil 1966;12:593-595. doi: 10.1530/jrf.0.0120593
11. Murphy BD, James DA: The effects of light and sympathetic innervation to the head on nidation in mink. J Exp Zool 1974;187:267-276. doi: 10.1002/jez.1401870210
12. Murphy BD, Concannon PW, Travis HF, et al: Prolactin: the hypophyseal factor that terminates embryonic diapause in mink. Biol Reprod 1981;25:487-491. doi: 10.1095/biolreprod25.3.487
13. Deng L, Chunjin L, Chen L, et al: Research advances on embryonic diapause in mammals. Anim Reprod Sci 2018;198:1-10. doi: 10.1016/j.anireprosci.2018.09.009
14. Papke RL, Concannon PW, Travis HF, et al: Control of luteal function and implantation in the mink by prolactin. J Anim Sci 1980;50:1102-1107. doi: 10.2527/jas1980.5061102x
15. Murphy BD: Precocious induction of luteal activation and termination of delayed implantation in mink with the dopamine antagonist pimozide. Biol Reprod 1983;29:658-662. doi: 10.1095/biolreprod29.3.658
16. Renfree MB, Tyndale-Biscoe CH: Intrauterine development after diapause in the marsupial *Macropus eugenii*. Dev Biol 1973;32:28-40. doi: 10.1016/0012-1606(73)90217-0
17. Rudd CD: Sexual behavior of the male and female tammar wallabies (*Macropus eugenii*) at post-partum oestrus. J Zool 1994;232:151-162. doi: 10.1111/j.1469-7998.1994.tb01565.x
18. Hinds LA, Tyndale-Biscoe CH: Plasma progesterone levels in the pregnant and non-pregnant tammar, *Macropus eugenii*. J Endocrinol 1982;93:99-107. doi: 10.1677/joe.0.0930099
19. Tyndale-Biscoe CH, Hearn JP: Pituitary and ovarian factors associated with seasonal quiescence of the tammar wallaby, *Macropus eugenii*. J Reprod Fertil 1981;62:225-230. doi: 10.1530/jrf.0.0630225
20. Hardy K, Spanos S: Growth factor expression and function in the human and mouse preimplantation embryo. J Endocrinol 2002;172:221-236. doi: 10.1677/joe.0.1720221
21. Bhatt H, Brunet LJ, Stewart CL: Uterine expression of leukemia inhibitory factor coincides with the onset of blastocyst implantation. Proc Natl Acad Sci USA 1991;88:11408-11412. doi: 10.1073/pnas.88.24.11408
22. Stewart CL, Kaspar P, Brunet LJ, et al: Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature 1992;359:76-79. doi: 10.1038/359076a0
23. Threadgill DW, Dlugosz AA, Hansen LA, et al: Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. Science 1995;269:230-234. doi: 10.1126/science.7618084
24. Mohamed OA, Jonnaert C, Labelle-Dumais K, et al: Uterine WNT/beta-catenin signaling is required for implantation. Proc Natl Acad Sci USA 2005;102:8579-8584. doi: 10.1073/pnas.0500612102
25. Cha J, Sun X, Bartos A, et al: A new role for muscle segment homeobox genes in mammalian embryonic diapause. Open Biol 2013;3:130035. doi: 10.1098/rsob.130035
26. Fenelon JC, Banerjee A, Murphy BD: Embryonic diapause: development on hold. Int J Dev Biol 2014;58:163-174. doi: 10.1387/ijdb.140074bm
27. Cha J, Dey SK: Cadence of procreation: orchestrating embryo-uterine interactions. Semin Cell Dev Biol 2014;34:56-64. doi: 10.1016/j.semcdb.2014.05.005