The history of the discovery of embryonic diapause in mammals

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Received 25 January 2018; Revised 2 May 2018; Accepted 5 May 2018

Abstract

The first incidence of embryonic diapause in mammals was observed in the roe deer, Capreolus capreolus, in 1854 and confirmed in the early 1900s. Since then scientists have been fascinated by this phenomenon that allows a growing embryo to become arrested for up to 11 months and then reactivate and continue development with no ill effects. The study of diapause has required unraveling basic reproductive processes we now take for granted and has spanned some of the major checkpoints of reproductive biology from the identification of the sex hormones to the hypothalamic–pituitary axis to microRNA and exosomes. This review will describe the history of diapause from its origins to the current day, including its discovery and efforts to elucidate its mechanisms. It will also attempt to highlight the people involved who were instrumental in progressing this field over the last 160 years. The most recent confirmation of mammalian diapause was in the panda in 2009 and there are still multiple mammals where it has been predicted but not yet confirmed. Furthermore, there are many questions still unanswered which ensure that embryonic diapause will continue to be a topic of research for many years to come. Note that there have recently been several extensive reviews covering the recent advances in embryonic diapause, so they will be mentioned only briefly here. For further information refer to Renfree and Shaw 2014; Fenelon et al 2014; Renfree and Fenelon 2017, and references therein.

Summary Sentence

The history of the discovery of embryonic diapause in mammals, the key breakthroughs, the current understanding, and future directions for the field.

Key words: diapause, uterus, blastocyst, comparative reproduction, pregnancy, seasonal reproduction.

Discovery: historical summary

Hunters in Europe, familiar with the deer they hunted and being keen observers, were the first to describe what we now know as embryonic diapause, a temporary arrest of embryo development, in the mid-1800s [1, 2]. William Harvey is sometimes credited with the discovery in 1651 after recording his observations of the Royal hunt, but he examined only the red deer Cervus elaphus and the fallow deer Dama dama, not the roe deer Capreolus capreolus, which happens to be the only unguulate known to have embryonic diapause (see [3] for a detailed discussion of Harvey’s observations and comment on Willis’s [4] mistranslation of “red” for “roe” deer in only one place). The hunters observed that there was a conundrum in the roe deer between observations of the timing of mating and the time of birth, as the pregnancy seemed to continue for far longer than expected [3]. Mating had been observed in July and August, but no embryo was visible in the tract until late December or early January [2]. Zeigler found no spermatozoa in the testis or the vas deferens by November, but still observed a 1–3 mm blastocyst in mid-December, and an elongating one by late December. He concluded that embryo development was retarded. In 1854, Bischoff [1] reiterated the puzzle:

“Es ist allgemein wohl bekannt, dass unter den Jagern . . . dass die Brunst im Juli und August eine falsche, die in Dezember wie wahre sie.” It is generally well known by hunters
...that the (apparent) oestrus (heat) in the months of July and August is a false one, and that the true time of oestrus is (occurs) in December.

Bischoff sampled 150 roe deer, and recovered blastocysts from the uterus between August and December, and concluded that the arrested development was due to an exceptionally slow growth of the blastocyst in the uterus (Figure 1). The state of the blastocyst was finally confirmed in 1899–1902 by Keibel [5, 6] and his student Sakurai in 1906 [7] who demonstrated that the stages of blastocyst development took 4–5 months in the roe deer, compared to 4–5 days in the sheep or pig. They also noted that there was some slow growth during the 4 months of delay with postimplantation development subsequently lasting 5 months. The uniqueness of the roe deer timing of mating separated by an extended period of time from active pregnancy was emphasized by a detailed comparison with the reindeer, a related species, in which delay does not occur [8]. The roe deer continues to be one of the best understood diapause species to this day [9, 10].

Contemporaneously, in 1873, Herbst [11] described the mating time and gestation in the Eurasian (European) badger, Meles meles, but it was Fries in 1880 [12] who first examined the actual embryos and fixed the approximate time of implantation. In 1931, Fisher [13] subsequently described the badger reproductive cycle in detail and confirmed implantation was delayed with a 6 month free vesicle stage, followed by an active development of about 2 months. Hamlett [14] later showed that the American badger Taxidea taxus had a similar duration of quiescence to the nine-banded armadillo Dasypus novemcinctus (see below). However, the detailed studies of badgers were not done until the work of Canivenc and Bonni-Laffargue in France and Harrison in the UK in the late 1950’s—early 1960s [15–18]. They noted that the corpora lutea are inactive during delay, and later, that there is an environmental (photoperiod and temperature) control of delay [19].

The next species in which diapause was suspected was identified by Lataste in 1888 (Figure 2) [20, 21] who described prolonged gestation in 5 species of lactating rodents (gerbils and mouse), but he did not examine any embryos. Lactational control of diapause is widespread amongst mammals, and in the rat and other rodents its duration depends on the number of young suckled [20, 22, 23]. Daniel in 1910 [24] and King in 1913 [25] confirmed the observations of Lataste in the mouse Mus musculus and rat Rattus norvegicus, but it was left to Kirkham in 1916 [26] to recover blastocysts from mice suckling young making it the fourth species (armadillos were the third species: see below) to have diapause confirmed by direct examination of embryos. Since these discoveries, lactational inhibition of implantation (or delayed implantation as Lataste termed it) has been described in many other rodents and is assumed to occur in the majority of rodent species. Mice have been a key species in defining the regulation of diapause and remain the best studied mammalian species.

The nine-banded armadillo was the third species for which embryological data provided confirmation of diapause. Armadillos are especially interesting since the single blastocyst forms four identical quadruplets by division of the embryonic disc [27]. In 1913, Patterson recovered armadillo embryos from the uterus and observed a period of quiescence of “about 3 weeks” before implantation [27]. Hamlett [28, 29] conducted a comprehensive study of the armadillo and established the duration of the free vesicle stage to be 14 weeks in both the armadillo and in the American badger. Hamlett noted that the corpus luteum of the armadillo was inactive during the whole of this time, and the lack of effect if it was removed, similar to the later observations on the Eurasian badger [30] (but note that bilateral ovariotomy of armadillos in delay does result in implantation [31, 32]). Carl Hartman had earlier attempted to define the physiological basis of diapause in the armadillo, but he comments that “this work would have been better left to Texans to solve on Texas armadillos in Texas” (see his Introduction to the first symposium on “Delayed Implantation” [33]). Diapause was also identified in a South American armadillo, Dasypus hybridus [34].

The last of the early discoveries of diapause was of diapause in the shrew, Sorex araneus in 1935. Brambell [35] was the first to observe lactating females with free uterine vesicles. Subsequently, embryonic diapause has been described in a number of insectivores. Only one species of the Siberian mole Talpa Altaica has diapause, along with multiple examples in the soricine but not in crocidurine shrews (reviewed by [36]). Their very short gestation length and long lactation period resembles that of marsupials somewhat.

Many carnivorous mammals show diapause. Several mammals kept in captivity for their fur (mustelids in particular) led to the next description of diapause. The American marten Martes americana, has an approximately 9 month delay (Ashbrook and Hanson 1927a, 1927b; 1930 in Hamlett [34]) whilst sable, Martes zibellina, mate in July and have a 7–7.5 month delay and mink Neovison vison have a diapause of up to 45 days [37, 38]. Black bears Ursus americanus, brown bears Ursus arctos, and polar bears Thalarctos maritimus all have a diapause of at least several months, delivering a highly dependent altricial young during hibernation [34]. The first embryos recovered from the uteri of black bears were not reported until Wimsatt’s [39] study in 1963. Interestingly, the most recent species in which diapause has been confirmed is the panda Ailuropoda melanoleuca in 2009 [40], although it was suspected by the Peking Zoo in 1974 (Wright in [41]) (Figure 3).

Another group that proved very challenging to study were the seals and sea lions. Bertram in 1940 [42] thought diapause occurred in harp seals Pagophilus groenlandicus, hooded seals Cystophora cristata, bearded seals Erignathus barbatus, and Weddell seals Leptonychotes weddellii, but this was confirmed much later. In a study that went from 1949 to 1962, the harbor and gray seals, Phoca vitulina and Halichoerus grypus, were shown to have diapause (reviewed by Harrison [43]). Diapause was established as a general rule for seals in 1951 by Pearson and RK Enders [44] working on the northern fur seals, Callorhinus ursinus. Joe Daniel was the first to undertake experimental manipulation of the northern fur seal, taking on the challenging task of trying to alter the cycle of a wild animal in a few weeks and collecting samples to understand its reproduction (reviewed in [41]).

Many bats show various forms of delay during pregnancy [45]. The African straw-colored fruit bat Eidolon helvum was the first to be described to have embryonic diapause in 1965 [46, 47]. Interestingly, Schreiber’s bent-winged bat, Miniopterus schreibersii, has also been shown to have diapause but the duration of the diapause is longer in the French population that spends longer in hibernation than in the Australian population [48, 49]. A number of bat species also exhibit a postimplantation delay where development is greatly slowed at the gastrulation stage [50, 51]. The Indian short-nosed bat, Cynopterus sphinx, has two successive pregnancies every year, but only one of these undergoes delayed development where pregnancy is extended by around 25 days [52, 53].

Today, we know that there are at least 37 species of marsupials with diapause, almost a quarter of the known diapause mammals. Macropodid marsupials have one of the highest incidences...
of embryonic diapause of any mammalian family with only three species confirmed to lack diapause (more details below). The existence of diapause was confirmed in marsupials much later than in other species [54, 55] even though it had been suspected by several researchers in the early 1900’s [56–58]. A slight diversion here is needed to explain why its discovery was so late. Studies on marsupials had been amongst the earliest studies of mammalian reproduction. Carl Hartman in the 1920s in the U.S.A. [59, 60], working on the North American opossum Didelphis virginiana, was the first to recognize the value of marsupials for comparative studies, and the first to use experimental approaches for investigating the endocrinology of reproduction. In Australia, JP Hill [61–63] also recognized the value of marsupials and discovered that the bandicoots (Peramelidae) have an allantoic as well as a yolk sac placenta. In 1913, Hill and O’Donoghue [64] investigating the estrous cycle and pregnancy in the native cat, Dasyurus viverrinus, showed that similar changes occurred in the uteri and mammary glands of unmated females, and coined the term “pseudopregnancy.” This term was adopted by Long and Evans [65] and Hammond and Marshall [66] to describe the appearance of pregnancy after a sterile mating in rats and rabbits, and is no longer used in marsupial reproduction. While Hill [56] and Flynn [58] reported the occurrence of concurrent pregnancy and lactation in the feathertail glider Acrobates pygmaeus (1900) and Tasmanian bettong Bettongia gaimardi (1930), marsupial research lagged after the 1920s–30s and around 30 years elapsed before Sharman’s 1955 [55] discoveries in the quokka Setonix brachyurus, reawakened interest in diapause and in marsupial reproduction. The results of those initial discoveries of diapause have led to one macropodid species, the tammar wallaby Macropus eugenii, becoming one of the three best studied diapause mammals about 50 years later [67], 150 years after the first discovery of diapause in the roe deer. Historically, the roe deer and the western spotted skunk Spilogale gracilis have been two of the most intensively studied diapause species whilst in recent times the best understood species, along with the tammar wallaby, are the mouse [68] and the mink [69]. Research on these species has provided much of the information that follows.
Challenges that needed to be met

By 1935, there were 7 confirmed mammal species with embryonic diapause and another 11 predicted [34, 56–58]. Between 1955 and 1976, there were over 250 publications on embryonic diapause [70], detailing diapause in a wide variety of unrelated mammalian families that now number over 130 species in 10 different orders [71]. Diapause is of particular interest because of its unique form of growth control and its reversibility. However, the study of diapause requires access to animals, some of which were not especially tractable for study, for example, seals and polar bears. Confirmation and characterization of diapause in a species requires extensive observations and analyses over an extended period that can be difficult for many of the species that are predicted to exhibit it. There are still multiple species for which diapause is predicted to occur but for which it has not been confirmed by detailed embryological studies, e.g. in the Burramyidae (pygmy possums) [72]. For each of the species studied, there were particular challenges specific to their situation as well as common challenges to be overcome for all species. Harrison [73] commented in 1969 on how difficult it is to catch pregnant seals, which are particularly cautious and apprehensive and often not easily accessible [41, 73]. At that time, experimental investigations to indicate how delay once initiated can be artificially terminated were not yet possible in pinnipeds [73]. Cross-species comparisons became invaluable but were not without their own set of challenges given the variations that diapause can take e.g. lactational versus seasonal, prolactin being stimulatory versus inhibitory to reactivation.

From 1843 to the 1930’s, investigations into embryonic diapause focused on confirming its presence and attempting to explain the external mechanisms by which it was induced. By the 1940’s, endocrine approaches to understanding how diapause was controlled had begun after the identification of the sex hormones. Weichart [74, 75] was the first to identify that the duration of delay in rodents correlated with the number of suckling young and that in vivo administration of estrogen induced reactivation and implantation in the rat. This was later confirmed in other species with the administration of progesterone able to reactivate marsupials [76, 77]. The influence of hormones was further confirmed by observations that ovariectomy can either induce diapause or reactivation depending on the species involved [31, 78, 79]. By this point, investigations into the external mechanisms controlling entry into and reactivation from diapause had also begun with the identification of both seasonal and lactational influences. Altering day length induced reactivation in the American marten [80], whilst removing the pouch young induced reactivation in the quokka, Setonix brachyurus [55].

The first symposium on embryonic diapause occurred in 1963 at Rice University, Houston, U.S.A., organized by Professor Alan C Enders and introduced by Carl Hartman [81]. This symposium brought together experts on marsupials, bears, mustelids, badgers, seals, mink, rat, guinea pig, and armadillo. The conclusion of that first symposium (by EC Amoroso) drew together the association of endocrine and environmental factors and the commonality of the physiological processes in the control of implantation, but it was unknown at the time whether these were mediated through the blastocyst or uterus, a question that is still not fully understood (see below).

It took another 17 years for the second symposium to be held in February 1980 at Thredbo, Australia (Figure 4) [82]. This symposium agreed to adopt the term “embryonic diapause” as originally suggested by Baevsky in the 1963 symposium instead of “delayed implantation,” which does not apply to all species. By this time, some of the complications involved in attempting to elucidate the mechanisms of embryonic diapause amongst all species were becoming apparent [51]. In contrast to all species examined previously, in vivo hormone administration did not reactivate the blastocyst in the mink, skunk, fur seal, or armadillo [41, 83, 84]. Furthermore, the tammar wallaby was described as the first mammal to exhibit both lactational and seasonal diapause [85]. Along with the tammar, studies on the skunk subsequently provided a great deal of detailed experimental data on the endocrine, photoperiodic, and lactational control
Figure 3. Timeline of initial species discovery of the potential for embryonic diapause to confirmation of diapause examples. Note for the nine-banded armadillo and the honey possum, confirmation of diapause was also the first identification of diapause. Roe deer, *Capreolus capreolus*, Eurasian badger, *Meles meles*, house mouse, *Mus musculus*, feathertail glider, *Acrobates pygmaeus*, nine-banded armadillo, *Dasypus novemcinctus*, Eurasian shrew, *Sorex araneus*, northern fur seal, *Callorhinus ursinus*, honey possum, *Tarsipes rostratus*, giant panda, *Ailuropoda melanoleuca*, and African straw-colored fruit bat, *Eidolon helvum* [1, 2, 5, 11–13, 24, 27, 35, 40, 46, 56, 136, 137] (Wright in [41]). Image credits: Karol Zub, Sara Evans, kallerna—own work, CC BY-SA 3.0, J. Patrick Fischer—own work, CC BY-SA 3.0.

of diapause (reviewed by [84, 86]). These studies demonstrated the importance of the corpus luteum and hypothalamic–pituitary control and began to dissect the critical role of melatonin in the regulation of seasonal diapause (see below).

By the 1970’s, the beginnings of embryo culture techniques allowed the first characterization of the diapause blastocyst. These investigations showed that in the mouse diapause blastocyst, cell division is arrested in the G1/G0 (but probably in the G0) phase of the cell cycle and embryo metabolism is depressed [87–89]. This decreased metabolism and cell cycle arrest (or greatly slowed cell cycle) have now been established as hallmarks of a diapause blastocyst in all mammals [90]. Concurrent with this were the observations that uterine secretions are low during diapause and increase at reactivation and in vitro hormones do not reactivate the blastocyst, providing the first indications of how hormonal control of the uterus influences the blastocyst [89, 91–94]. Previously, the significance of uterine control had been highlighted by Tyndale-Biscoe in 1963 [95] who showed that transfer of diapause blastocysts to a reactivated uterus induces reactivation and vice versa.

The other major advance of this time was in identifying how the photoperiodic or lactational signal is transmitted and what neural and pituitary hormones are involved in multiples species. This included identification of the importance of prolactin secreted from the pituitary in influencing luteal function, the relationship between the corpus luteum and progesterone, how seasonal control of diapause requires intact photoreceptors and the pineal gland, and how lactational diapause is influenced by the direct suckling of the young affecting the neural control of prolactin secretion [96–102]. Interestingly, however, the effects of prolactin differed between the macropods and the mustelids. In the mustelids, administration of prolactin results in reactivation whilst in the macropods, administration of prolactin maintains diapause [84, 103, 104]. Consistent with this, hypophysectomy during diapause in the mustelids results in a decrease in progesterone and a failure of reactivation whilst in macropods it results in reactivation [105–108]. Finally, observations that a nondiapause species blastocysts (ferret) can be induced to undergo diapause in a mink uterus (a closely related diapause species) were the first indications that the control of diapause may be a universal mechanism amongst all mammals [109].

Since the second symposium, there have been significant breakthroughs in the molecular control of embryonic diapause. In particular, advances in understanding the importance of uterine secretions, their identification, and their potential functions in controlling the state of the embryo. In the tammar wallaby, uterine protein synthesis increases about 1 day before increase in blastocyst metabolic activity, and a recent proteomics study revealed that 21% of the protein content of uterine fluid was composed of secretory proteins [110, 111]. The first factor to be identified as specifically expressed at reactivation in the uterus and downregulated during diapause was leukemia inhibitory factor (LIF) in 1991, whilst the first factors to be differentially expressed in the blastocyst between diapause and reactivation were members of the transforming growth factor-β family in 1992 [112, 113]. Since then, levels of multiple growth factors and cytokines have been observed to be differentially expressed in both the uterus and blastocyst with the majority having decreased
levels during diapause that increase at reactivation (for review see [90]). Indications that the blastocyst also takes a proactive role in its own development were first identified by the presence of heparin-binding epidermal growth factor (HB-EGF) specifically in the uterus surrounding the blastocyst at reactivation, several hours before implantation commenced [114]. This was later confirmed by the ability of HB-EGF to induce its own expression in the uterus [115].

Many of the above growth factors and cytokines have now been confirmed to be conserved amongst mammals [90]. Members of the EGF family were identified as being specifically expressed during reactivation in both the mink and the tammar wallaby as well as the mouse [116]. Similarly, LIF is increased at reactivation in the tammar wallaby, mink, mouse, and western spotted skunk [112, 117–119, Renfree et al unpublished]. This is despite a report that prolactin stimulates LIF expression in the skunk and prolactin is inhibitory to reactivation from diapause in the tammar wallaby [104, 118]. Furthermore, in the mouse, mink, and tammar wallaby uterus, the muscle segment homeobox genes MSX1 and MSX2 are specifically expressed during diapause and downregulated at reactivation, and in the mouse MSX is essential to maintain diapause [104, 118]. These results suggest that regardless of the hormonal control involved in the uterine control of embryonic diapause, many of the molecular controls between the uterus and the blastocyst have been conserved between species.

From studying potential control factors at the individual level came, the advent of genomic and proteomic technologies that allowed for investigation into the comparison between diapause and reactivation to occur at a large scale. This has revealed an abundance of potential new targets for future research, some of which like the polyamines, have been confirmed to have a significant role in the control of diapause. In the mink, a suppressive subtractive hybridization study identified 123 genes that were differentially expressed in the uterus at reactivation compared to diapause and included three members of the polyamine synthesis pathway [122]. It was subsequently shown that inhibiting polyamines can maintain diapause in both the mink and mouse [123, 124]. In the mouse blastocyst, Hamatani [115] identified 80 genes that were highly expressed during diapause and 149 that were highly expressed at reactivation. Similarly, 91 genes were identified as upregulated in the mink blastocyst at reactivation [126]. In addition, a recent proteomic study identified 277 proteins that were specifically expressed in the diapause mouse blastocyst and 585 proteins specifically expressed at reactivation [125]. The specific roles of the majority of these factors in diapause are currently unknown.

Finally, recent embryo transfer experiments have posed new questions about the origin of diapause. A small number of sheep embryos, transplanted to a diapause mouse uterus, enter into "diapause" and can reactivate when transplanted back and survive to birth, suggesting that embryonic diapause may be a universal mechanism amongst all mammals [127]. However, it may not have been a true diapause but simply a lack of sufficient nutrients and growth factors to undergo expansion, given the few blastocysts of those transferred that resumed growth. Further experiments are needed to explore this.
Figure 5. Outline of blastocysts during diapause to show the relative sizes of vesicles and inner cell masses in a range of diapause species. The badger, honey possum, and roe deer undergo a period of slow growth during diapause, sizes shown here are the maximum. Note, the tammar and honey possum do not have an ICM. Scale bar = 1 mm (adapted from Hamlett [34].

**Current state of the art, questions that remain & future directions**

It is unknown why the phylogenetic distribution of embryonic diapause is so broad. There are probably many more diapause species than have been described so far, especially rodents (for review see [71]). There are no obvious commonalities amongst the species that exhibit it and no correlation between physiological aspects such as size of animal and blastocyst size (Figure 5). The size of the blastocyst in diapause is variable amongst species and in most, cell division completely halts, but in a few species like the roe deer, armadillo, honey possum, and badger, the blastocyst expands slowly. If there are molecular commonalities amongst all mammals, it is possible that either diapause was the ancestral state or diapause has evolved independently multiple times by taking advantage of common molecular checkpoints already in place, e.g., implantation. Many aspects of the initial implantation process appear conserved across mammals [71]. However, as discussed by Wimsatt [51], it is essential that given the diversity of strategies available that we do not restrict the study of implantation (and embryonic diapause) to just a few species as this prevents us from distinguishing between those regulatory mechanisms that are conserved and those that are species-specific.

In addition to the downregulation of growth factors, there are a number of upregulated blastocyst genes that are induced during diapause and have a potential active role in maintaining the diapause state [115, 116]. At reactivation, the blastocyst can signal the uterus to modulate endometrial responsiveness and subsequent implantation [115, 128, 129]. It is clear now that a two-way molecular communication between the uterus and the blastocyst is required to induce, maintain, and reactivate from embryonic diapause. Multiple interconnecting factors appear involved in an increasingly complex and time-dependent network [90]. Furthermore, there are likely multiple essential factors yet to be identified including microRNAs [130]. Recent proteomic studies have also highlighted a wealth of potential new candidates [110, 125]. The challenge remains in identifying the minimum factors required and their functions. There is evidence that many of the factors are conserved amongst diapause species and interspecies comparisons between evolutionarily distant species are likely to be very informative.

For example, inhibition of polyamines maintains diapause, including the blastocyst cell cycle arrest but it is unknown to what extent their inhibition mimics the natural diapause state [123, 124]. There are currently no known reliable molecular markers that characterize a blastocyst in diapause, beyond the canonical cell cycle arrest and depressed metabolism [90]. Such markers are essential, along with improved culture methods, to test potential stimuli and inhibitors. As mentioned above, many of the molecular mechanisms are likely conserved amongst diapause species. Identification of specific markers of diapause (and reactivation) would also identify to what extent the molecular mechanisms are conserved amongst species and whether this extends to the potential for all mammalian embryos to enter into a diapause state. With the advent of new molecular advances over the past 10 years, we now have the tools that will allow us to begin to unravel these questions.

The remarkable thing about diapause is that cell division is halted, but the condition is reversible. Understanding how this is controlled at a molecular level has a multitude of potential applications, such as improving viability of blastocysts in assisted reproductive technologies, in the derivation of embryonic stem cells, and the identification of novel cancer therapies to halt or slow cell division. Understanding embryonic diapause can provide further insight into the minimum requirements required to keep an embryo alive for an extended period of time with no ill effects and determine the factors produced by the embryo to communicate with the uterus. For assisted reproductive technologies, this would potentially provide options to improve embryo culture media, provide an alternative to cryopreservation, and identify novel noninvasive markers of embryo health for embryo selection prior to transfer. This could also provide novel contraceptive targets. Stem cells were first derived from the inner cell mass (ICM) of diapause blastocysts [131]. Curiously, it was previously identified that mouse trophoblastic vesicles can enter into diapause in the absence of ICM, but the ICM did not enter into diapause in the absence of the trophoblast [132]. Recently, two studies showed how inhibition of two downstream signaling factors myelocytomatosis oncogene (MYC) and mechanistic target of rapamycin can induce a reversible arrest in both the mouse blastocyst and mouse embryonic stem cells (ESCs) [133, 134]. Furthermore, the transcriptomic profile of arrested ESCs resembled that of a diapause epiblast [133]. Finally, in essence, embryonic diapause is the study of how to stop and restart prolific cell growth. The reactivation of the embryo, including its rapid resumption of cell proliferation and increased metabolism followed by its subsequent implantation, has many similarities to the invasive nature of cancer cells [135]. Preventing either “reactivation” of a cancer cell or inducing it to enter into “diapause” could provide novel future treatment therapies for either inoperable tumors or prevent metastasis.

**Conclusions**

Protection of the mammalian young can be achieved in many ways, including extending either intrauterine pregnancy or the period of maternal nutrient provisioning by lactation. A key innovation in a subset of mammals was the use of embryonic diapause to
significantly extend pregnancy until conditions are ideal for birth and postnatal survival. The study of diapause has had a significant role in the history of research in reproduction. The future of diapause also holds much promise for reproduction research. It is now only that we have the tools available to begin to elucidate the complexities involved in diapause and the molecular cross-talk that occurs between the preimplantation embryo and uterus. Furthermore, understanding the evolution of diapause will provide new insights not only into the evolution of viviparity but will have significant implications for seemingly unrelated areas, such as assisted reproduction technology and unrestrained growth as occurs in cancer. There are still many questions to be answered and new species to be studied. Diapause will continue to be a vigorous topic of research.

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