

## OPINION

# Do human concepti have the potential to enter into diapause?

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**Although there is no direct evidence as to whether human concepti have the potential to enter into diapause before implantation, the possibility that human concepti may be capable of following this developmental pathway if exposed to an appropriate environment cannot be ruled out. Direct evidence remains elusive because of the ethical restraints associated with research activities within this area of knowledge. If conceptus diapause has evolved in primates and persists at the present time despite its apparent limited or no adaptive advantage, artificial induction of diapause in humans may have clinical implications for increasing: (i) the viability of concepti after biopsy, freezing–thawing or any other experimental procedure that tends to decrease cell numbers or division rate of concepti; and (ii) the relatively low implantation rates obtained at the present time after uterine transfer of human concepti fertilized *in vitro*. Furthermore, conceptus diapause may be a good paradigm to understand the interplay between the different genetic/molecular components of both the conceptus and endometrium at implantation.**

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The role played by the uterus in the control and regulation of preimplantation conceptus development in mammals is demonstrated by a fascinating, although apparently forgotten, reproductive strategy called 'conceptus diapause'. When this pathway is taken, mammalian concepti enter into a quiescent state at the blastocyst stage, with no mitotic figures or they expand at a very slow rate for a period of several days, or even months (Renfree and Calaby, 1981; Sandell, 1990; Mead, 1993). Although, during this stage, blastocysts are actually in

a high energy state (i.e. a high ATP/ADP ratio) (Nieder and Weitlauf, 1984), they have reduced levels of metabolic and synthetic activity and become activated only in response to a change in the maternal endocrine status. In some species (bats and insectivores, armadillos, roe deer and carnivores) such a period of conceptus diapause occurs as an obligatory (seasonal) part of pregnancy; in others (marsupials, insectivores and rodents) it may or may not occur depending on conditions in the maternal environment (facultative diapause). For example, in mice (Bindon, 1969) and rats (Zeilmaker, 1964), mated at post-partum oestrus, delay of implantation results from the suckling stimulus of the newborn young. A similar delay in implantation can be induced by a number of experimental techniques, including transfer of blastocysts to oviducts of immature mice (Papaioannou, 1986), ovariectomy, hypophysectomy or administration of neuro-depressive drugs during the first days of pregnancy (Gidley-Baird, 1981).

Conceptus diapause has been described in ~100 mammalian species distributed across eight orders and 17 families (Sandell, 1990). It is important to note, however, that very closely related pairs of species or even subspecies may differ in this reproductive strategy, with some exhibiting diapause and others not (Renfree and Calaby, 1981). Failure to detect conceptus diapause in a particular species may be explained either by the incapacity of concepti to enter a dormant state, or by the scarce attention paid to this reproductive trait. In addition to this, in the human, we have to consider the ethical restraints associated with research in this area. These circumstances may explain the fact that, despite human reproduction attracting the attention of many philosophers, scholars and scientists since the ancient Greek era, no direct evidence has yet been reported on whether human concepti have the potential to enter diapause before implantation. A few studies have described early uterine (Naaktgeboren *et al.*, 1986; Hamori *et al.*, 1989) and ectopic (Beghin *et al.*, 1992; Korhonen *et al.*, 1996) pregnancies exhibiting a delay in implantation, as measured by delayed appearance of human chorionic gonadotrophin (HCG), even though this phenomenon may be rare. However, the short delay in the appearance of HCG (<5–6 days) observed in these studies is more compatible with a slowing of development, rather than with a temporary diapausic arrest. It is known, for instance, that the appearance of serum HCG in pregnancies after in-vitro fertilization (IVF) in stimulated cycles shows a delay of 3–4 days, in comparison with pregnancies obtained after in-vivo fertilization in either natural or stimulated cycles (Englert *et al.*, 1984). Small

discrepancies in the timing of appearance of serum HCG between women may be due, among other factors, to transient differences in developmental rates of concepti imposed by the stressful/abnormal conditions that concepti face *in vitro*, in the Fallopian tubes or in an asynchronous/unfavourable endometrium.

Although the studies mentioned above fail to provide clear evidence for the potential of human concepti to enter into diapause, another study (Grinsted and Avery, 1996) shows, although indirectly, that the phenomenon of conceptus diapause may occur in humans. In that study, an implantation delay of up to 5 weeks was recorded after IVF of 11 oocytes and uterus transfer of three concepti in a 36 year old woman who had previously delivered four children after natural conceptions. Subsequent ultrasound scans showed that the fetus progressed normally, but was delayed by 5 weeks compared with the oocyte aspiration. It is interesting to note that the third and fourth deliveries were delayed by 5 weeks and 2 weeks, respectively, based on the date of the last menstruation. Furthermore, in contrast to other studies (Naaktgeboren *et al.*, 1986; Beghin *et al.*, 1992), menstruation was not restored after the conception cycle. This fact suggests that the mechanism which induced delayed implantation in this study (Grinsted and Avery, 1996) may be different to that causing the short delay in the appearance of HCG observed in the other studies.

Further evidence suggesting that human concepti may enter preimplantation diapause comes from pregnancies resulting in fetuses/babies with intrauterine growth retardation, i.e. small (SGA) or very small (VSGA) for gestational age. Although the real causes of intrauterine growth retardation are quite difficult to ascertain because of the multifactorial condition of this trait (there are at least 14 factors with a well-established direct causal impact on intrauterine growth, and four factors for gestational duration; for review, see Kramer, 1987), the possibility that some SGA or VSGA fetuses/babies result from pre-implantation diapause cannot be ruled out. In particular, we should mention those SGA or VSGA fetuses/babies of unknown aetiology exhibiting proportional reductions in weight, length and head circumference.

According to an earlier review (Mead, 1993), the physiological/hormonal mechanisms controlling conceptus diapause are very different between the various orders of mammals showing this reproductive trait. For instance, ovariectomy causes the death of blastocysts in mustelids (order Carnivora), but reactivates a diapausing conceptus and induces implantation in the armadillo (order Edentata). For this reason, it would be hazardous to extrapolate data from one order to another. However, this rule does not likely apply for species belonging to the same family. In the comprehensive review by Mead (1993), it can be noted that different genera/species belonging to the same family such as the European badger, spotted skunk or mink within the family of Mustelidae; the tammar wallaby, quokka, red-necked wallaby or red kangaroo within the family of Macropodidae; and the laboratory mouse and rat within the family of Muridae, use similar physiological/hormonal mechanisms to control conceptus diapause. Although there may be exceptions, this rule is quite reassuring, especially to

encourage experimentalists to develop a primate model to study the physiological/hormonal mechanisms controlling conceptus diapause in human beings.

As a first step in the testing of the hypothesis that primates have the potential to enter into diapause, it would be interesting to ascertain whether primate concepti can enter into a diapause arrest when transferred to the uterus of a foster breast-feeding female. We should bear in mind that in many regions of the world, women breast-feed one child while becoming pregnant with the next. Thus, many implantations from reactivating diapause blastocysts may pass unnoticed or be mistaken as resulting from later ovarian cycles, in particular, if ovulation and conception take place during the period of lactational amenorrhoea. Techniques and methods of artificially inducing conceptus diapause in primates should be also developed. For instance, the effects of maternal administration of antiprogesterins such as mifepristone (RU486) and/or prostaglandins on conceptus cleavage rates and development during the peri-implantation period could be analysed. In fact, administration of diclofenac, a prostaglandin synthesis inhibitor, or RU486 along with misoprostol (a prostaglandin E<sub>1</sub> analogue) may delay implantation for a period of up to 6 days, as measured by a rise in blood monkey chorionic gonadotrophin (mCG) in rhesus monkeys (*Macaca mulatta*) (Nayak *et al.*, 1997). This delay may be caused by a drug-induced retarded endometrial maturation as well as a diapause arrest of blastocysts. It would be also worth determining the effects of administering neuro-depressive drugs during the first days of pregnancy on blastocyst development and implantation. This procedure has been shown to be efficient in inducing delayed implantation in rats (Shani *et al.*, 1979). Furthermore, Grinsted and Avery (1996) reported that the woman exhibiting an apparent 5-week delay in conceptus implantation felt very depressed about not being pregnant after conceptus transfer, i.e. during the period that the conceptus presumably was induced to enter into a diapause arrest.

If human concepti exhibit some growth during diapause as occurs in roe deers, rats, mice, bears, seals and mustelids (Renfree and Calaby, 1981), the artificial induction of diapause may be used to increase the viability of concepti after biopsy, freezing–thawing or any other experimental procedure that tends to decrease cell numbers or division rate of concepti (Tarín and Handyside, 1993). In this context, another study (Kaufman *et al.*, 1977) is worth noting, in which a period of induced implantation delay to allow concepti to reach higher cell numbers before implantation had a beneficial effect on the development of diploid parthenogenetic concepti (they reached the forelimb bud stage, the most advanced diploid parthenogenetic conceptus observed to date). This is a similar stratagem to that used in another study (Tsunoda and McLaren, 1983), where better development of concepti was obtained by using serial, asynchronous transfers to pseudopregnant females to extend the overall pre-implantation time by 3–4 days and allow regulation of cell numbers before implantation.

Induction of conceptus diapause may be useful to reduce or minimize the potential damage inflicted by the freezing–thawing procedure to the mitotic apparatus of blastomeres during cell division. In fact, diapausing mouse concepti arrest

in the G<sub>1</sub> phase of the cell cycle (Sherman and Barlow, 1972), i.e. when no spindle microtubules can yet be seen. Furthermore, artificial induction of conceptus diapause may raise the relatively low implantation rates obtained at the present time after uterine transfer of IVF human concepti. It has been known for a long time that ovarian stimulation with exogenous gonadotrophins is associated with decreased endometrial receptivity to the conceptus (Paulson *et al.*, 1997; McKiernan and Bavister, 1998). Thus, in order to avoid this handicap, concepti may be artificially induced to enter into a quiescent state within the maternal uterus until conditions become more favourable for implantation.

If conceptus diapause has evolved in primates, and persists at the present time despite its apparent limited or no adaptive advantage, benefits from controlling diapause in primates/humans may be also reflected in the basic side of the research coin. Conceptus diapause may be a good paradigm to understand the interplay between the different genetic/molecular components of both the conceptus and endometrium at implantation. It is interesting to note that mouse concepti decrease the secretion of some stage-specific proteins as they enter into diapause, whereas reactivating concepti increase the secretion of these particular proteins (Weitlauf, 1994). The identification of these proteins and other cellular compounds as well as the mechanisms regulating their synthesis and the transcription of their respective coding genes may be fundamental to untangle the molecular machinery displayed at implantation by the conceptus and the endometrium. This is certainly a fascinating research area that needs renewed attention to emerge from its present diapausic arrest.

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