**—Mini Review—**

# *Differential Interactions between Embryo and Uterus during Implantation in Laboratory Animals*

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***Abstract:*** *Synchronous embryonic development and uterine differentiation is crucial to successful implantation and to pregnancy outcome; reciprocal interactions between the implantation-competent blastocyst and receptive uterus are necessary for successful implantation. Implantation involves the interplay of numerous signaling molecules, and the process is complicated and varies across species. Therefore, investigations into embryo and uterus crosstalk, including comparative research among species are necessary to improve reproductive biomedicine and animal husbandry. We herein focus on species-specific morphological changes, hormonal control, and molecular interactions that occur in the uterus and embryo during implantation in laboratory animals.*

***Key words:*** *Implantation, Embryos, Blastocysts, Uterus, Laboratory animals*

## Introduction

Implantation is a complex process involving spatiotemporally regulated endocrine, paracrine, autocrine, and juxtacrine modulators that mediate cell-cell and cell-matrix interactions. The implantation process varies among species, thus precluding the formulation of a unified theme. Successful embryo implantation is dependent on cellular and molecular crosstalk between the uterus and the embryo. The dynamic coordination of the endocrine, cellular, and molecular events via paracrine, autocrine, and/or juxtacrine processes produces within the uterus a favorable environment, the receptive state, which supports implantation. The embryo is also an active unit with its own molecular program of cell growth and differentiation. Therefore, deficiencies in uterine receptivity, embryo development, or the embryo-uterine dialogue compromise fertility. This review focuses on the species-specific morphological and molecular interactions underlying implantation in laboratory animals.

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## Morphological Studies of Species-Specific Implantation

Implantation is the process by which the blastocyst comes into intimate physical and physiological contact with the uterine endometrium. It has been divided into three stages: apposition, adhesion, and penetration [1, 2]. The first sign of attachment during implantation, the apposition stage, occurs on the evenings of days 4 and 5 of the pregnancy in the mouse and rat, respectively, and on day 6.5 in the rabbit [3–5]. In contrast, embryos implant late in the morning of day 4 in hamsters [6].

On the basis of different types of blastocyst-uterine cell-cell interactions, Bonnet proposed three categories of implantation—central, eccentric, and interstitial [7]. Central implantation occurs in mammals, such as rabbits, ferrets, and some marsupials. In these animals, blastocysts grow and expand extensively before implantation. However, the blastocysts of mice, rats, and hamsters are small and expand modestly. The implantation chamber in these species is formed by the invagination of the uterine epithelium, which is a characteristic of eccentric implantation. Interstitial implantation occurs in guinea pigs, chimpanzees and humans, and is characterized by the embedding of blastocysts within the subepithelial stroma.

Schlafke and Enders classified implantation into

intrusive, displacement, and fusion based on ultrastructural studies [8]. Intrusive implantation occurs in guinea pigs and humans and involves trophoblast penetration through the luminal epithelium that reaches and extends through the basal lamina. Displacement implantation occurs in rodents, and in this process the luminal epithelium is freed from the underlying basal lamina, facilitating the spread of trophoblasts through the epithelium. Fusion implantation, in which trophoblasts connect to the luminal epithelium by forming symplasma, occurs in the rabbit.

In many rodents, including mice and rats, implantation always occurs at the antimesometrial side of the uterus. In other animals, the embryos elongate and either attach over the entire endometrium (horse, pig, and wallaby) or only at specialized areas called caruncles (cow and sheep) [9]. Mice blastocysts are oriented with their inner cell mass (ICM) directed mesometrially, whereas in humans the ICM is directed antimesometrially. The mechanisms directing the blastocyst toward the antimesometrial luminal epithelium or orienting the blastocyst at the time of implantation remain elusive.

## Involvement of Steroid Hormones in Species-Specific Implantation

In all eutherian mammals, the major factors that specify uterine receptivity are the ovarian steroids progesterone and estrogens. Ovarian progesterone and estrogen are crucial for implantation in mice [10, 11] and rats [12], but ovarian estrogen is not essential for implantation in hamsters [13, 14], guinea pigs [15], rabbits [16], and pigs [17]. Some of these progesterone-dependent species may also require estrogen for implantation. The blastocysts of hamsters [18], rabbits [16], and pigs [17] may be capable of producing estrogen, but whether embryonic estrogen plays a role in implantation in these species is debatable. Recent evidence suggests that hamster blastocysts express the aromatase enzyme [6]. The mouse embryo lacks the aromatase activity that is necessary for estrogen synthesis [19].

The coordinated actions of progesterone and estrogen that regulate proliferation and/or differentiation of uterine cells in a spatiotemporal manner in mice and rats establishes the window for implantation [20]. For example, on the first day of pregnancy (as indicated by a vaginal plug) in mice, preovulatory estrogen secretion induces proliferation of uterine epithelial cells, and rising levels of progesterone secreted from freshly formed corpora lutea initiate stromal cell proliferation from day 3 onward. The stromal cell proliferation is further stimulated by a small amount of ovarian estrogen secreted on the morning of day 4 of pregnancy. These coordinated effects of progesterone and estrogen stop uterine epithelial cell proliferation and initiate differentiation [20]. During normal pregnancy, an active blastocyst in the uterus stimulates implantation. After attachment is initiated on day 4 at 2400 h, stromal cells surrounding the implanting blastocyst begin to proliferate extensively and differentiate into decidual cells (decidualization) [21].

## Delayed Implantation

Delayed implantation is a process in which implantation is postponed for a period of time. This causes the uterus to remain quiescent and an embryo in the blastocyst stage to become dormant. Delayed implantation occurs in many vertebrate species, but the underlying mechanisms directing delayed implantation are different in the various species that have adapted to this reproductive strategy [22].

In mice and rats, an ovariectomy before the presumed estrogen surge in the morning of day 4 of pregnancy prevents implantation and initiates blastocyst dormancy within the uterine lumen [3, 23]; this delayed implantation can be maintained for many days by continued treatment with progesterone. The process of implantation with blastocyst activation can be rapidly initiated by a single injection of estrogen in the progesterone-primed uterus [3, 23]. Delayed implantation does not occur in some species, such as the hamster, guinea pig, rabbit, and pig. Delayed implantation in mice is used as a model for understanding the molecular signaling originating from the embryo that influences uterine biology.

## Determinants of Blastocyst Competency Revealed Using the Delayed-implantation Mouse Model

For successful implantation in the receptive uterus, the blastocyst must also attain implantation competency. The first evidence that blastocyst activity determines the window of implantation in the receptive uterus was seen in reciprocal blastocyst-transfer experiments in a delayed-implantation mouse model [10, 24]. This model is a powerful tool for defining the molecular signaling components that direct blastocyst activation or dormancy. Using this model, a global gene

expression study showed that these two different blastocyst physiological states can be distinguished at the molecular level, and that the genes involved controlled the cell cycle, cell signaling, and energy metabolism [25]. The study also showed an upregulation of *Hbegf* expression, which encodes heparin-binding EGF-like growth factor (HBEGF) in activated blastocysts. This finding is complementary to earlier reports of upregulation of the HBEGF receptors ErbB1 and ErbB4 in blastocysts [25–27].

Other signaling molecules also participate in blastocyst dormancy and activation. Some data suggest that catecholoestrogens that are produced from primary estrogens in the uterus activate blastocysts [28]. Another lipid-signaling molecule that targets blastocysts is the endocannabinoid anandamide, which activates the G-protein-coupled cannabinoid receptors CB1 and CB2. Expression of *Cb1* in the trophectoderm and uterine synthesis of anandamide indicate that endocannabinoid signaling is crucial for implantation in mice [29–31]. Levels of uterine anandamide and blastocyst CB1 are coordinately downregulated with the attainment of uterine receptivity and blastocyst activation, respectively, but are elevated in the nonreceptive uterus and dormant blastocyst [29, 32, 33]. Indeed, implantation is postponed in wild-type mice in which the *in vivo* level of exogenously administered cannabinoid ligands is sustained, and this delay depends on the expression of CB1 receptors on the embryo [33]. Anandamide regulates blastocyst function by differentially modulating mitogen-activated protein kinase (MAPK) signaling and $Ca^{2+}$ channel activity via CB1 [32]. This is consistent with findings that MAPK and phosphatidylinositol 3-kinase/$Ca^{2+}$ signaling cascades are crucial to blastocyst development and activation [34–37].

## Categories of Functional Factors Influencing Implantation in the Mouse

Apposition and attachment are key steps in implantation and depend on synchronous development of blastocyst implantation competency and uterine differentiation into the receptive stage. Ovarian estrogen and progesterone, acting through their cognate nuclear receptors, influence several locally produced growth factors, adhesion molecules, cytokines, transcription factors, and vasoactive mediators and their receptors in the uterus and/or blastocyst to coordinate blastocyst-uterine crosstalk. This crosstalk further influences some of the signaling pathways to ensure the successful execution of the implantation process [38]. Functional factors are categorized into transcription factors, soluble mediators, and adhesion molecules [39].

### Cross-linker Proteins and Adhesion Molecules

Adhesive signaling systems are required for the attachment phase of implantation. Indeed, numerous glycoproteins and carbohydrate ligands and their receptors are expressed in trophectoderm cells and luminal epithelium around the time of implantation [39, 40]. CD44 is likely involved in periimplantation interactions. It recognizes polyanionic glycans including hyaluronan and chondroitin sulfate [41]. Furthermore, CD44 integral membrane proteins that cross-link with actin filaments by ezrin/radixin/moesin (ERM) proteins in the organization of cortical actin-based cytoskeletons, including microvilli formation [42]. Radixin and ezrin are involved in cellular organization of the trophectoderm during blastocyst activation prior to implantation, and radixin is particularly involved in preparing the mural trophectoderm (the presumptive site of attachment with the luminal epithelium) for implantation [43]. In contrast, the ERM-associated adhesive molecules CD44, CD43, ICAM-1, and ICAM-2 are present in the trophectoderm of dormant blastocysts. This suggests that, in dormant blastocysts before activation, adhesive molecules associated with ERM proteins are already positioned in a cell-specific manner for interacting with radixin and ezrin expressed in activated blastocysts [43]. Thus, ERM proteins expressed on trophectoderm cell surfaces of implantation-competent blastocysts may act as cross-linkers between actin and adhesive molecules and change the cell polarization and/or differentiation for adhesion and attachment with the luminal epithelium.

## Conclusion

Implantation is complicated and varies across species. Therefore, the formulation of a unified model for the molecular basis of implantation in mammals seems unrealistic at present. Mouse models have contributed significantly to our understanding of the numerous molecular and genetic mechanisms underlying implantation. Indeed, many gene-knockout mouse models have provided a wealth of information. However, key genetic technology, such as targeted mutation of specific genes, is not available for the other mammalian species because functional embryonic stem

(ES) cells cannot be produced with germline competence. This stresses the importance of careful interpretation of data obtained from mouse models concerning mechanisms of implantation and that have implications for human fertility and livestock production. Despite all the research to date, clearly further investigation into embryo and uterus crosstalk is necessary, and this includes comparative research in different species.

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