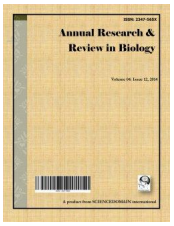

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# Role of Adrenomedullin in Trophoblast Invasion

**Kanchi Ravi Padma[1] and Penchalaneni Josthna[1*]**

*[1]Department of Biotechnology, Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati, Andhra Pradesh, India.*

***Authors' contributions***

*This work was carried by Author KRP. Author KRP designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.*

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## ABSTRACT

The Adrenomedullin peptide hormone has a potent vasodilatory activity. Nevertheless this novel peptide has exposed to be almost a ubiquitous peptide, with the many number of tissues and cell types synthesizing adrenomedullin. Adrenomedullin (ADM) and its related family peptides are calcitonin gene-related peptides (α and β-CGRPs), and intermedin/adrenomedullin-2 (IMD/ADM2) which play vital role as regulators of vascular tone and cardiovascular advances in vertebrates. Current research into their functions in reproduction has acknowledged the function of these peptides and their cognate receptors (calcitonin receptor-like receptor/receptor activity-modifying protein (CLR/RAMP) receptors) in fetal cum maternal blood circulation, feto-placental and uteroimplantation development along with that of female gamete development as well as gamete movement in the oviduct. Moreover, recent findings have enlightened the novelty, potential opportunities for the deterrence and treatment of aberrant pregnancies such as pregnancy-stimulated hypertension, preeclampsia, and IUGR. Conversely, chief efforts are still required to clarify the relationships between evident components of the CLR/RAMP signaling pathway and aberrant pregnancies before CLR/RAMP receptors can develop targets for clinical management. With this comprehension, this review summarizes current progression with specific focus on role of adrenomedullin during early implantation.

**Corresponding author: E-mail: penchalanenijosthna@gmail.com;*
*E-mail: thulasipadi@gmail.com;*

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## 1. INTRODUCTION

A Novel vasoregulatory peptide was discovered when scientists were screening for panel of peptides from a pheochromocytoma. At the same time they were looking for biological activity by testing whether the peptides could raise platelet cAMP levels. To their knowledge found a peptide with this activity. Later they purified and sequenced it, and named it "adrenomedullin" as it was found from the adrenal medulla. Kitumara et al., 1993 was the first to study on adrenomedullin its connection with Apoptosis or programmed cell death. Since ADM plays a vital function to the development and homeostasis of human tissues, including the human placenta. During normal pregnancy, cytotrophoblasts (CTs) and syncytiotrophoblasts (STs) were be regarded to be in a steady state; though it is likely that during placental attack there can be alteredness in this relationship, possibly by changing trophoblast cell turnover [1]. Trophoblast invasion into the uterine endometrium is crucial step for placentation [2]. In our previous investigations have demonstrated that trophoblast apoptosis and also have revealed elevated levels of placental apoptosis which resulted in intrauterine growth restriction (IUGR) and preeclampsia (PE) [3,4]. For the reader's advantage, we focused on apparent roles of these peptides and receptors based on recent studies [5]. Additionally, readers could check to a recent review by Lenhart and Caron that specifically focuses on the role and functions of ADM during pregnancy [6].

## 2. THE ROLE OF RAMPs IN THE VASCULATURE

Investigation on Genetic studies provided evidence that the ADM1 receptor (CLR/RAMP2) complex mediates numerous of the *in vivo* effects of hADM, principally in the vasculature. In humans, maternal levels of hADM are elevated from gestation and throughout pregnancy, with intensity level returning to basal 24 hours after delivery [7,8]. A hypoxic environment during the first trimester of pregnancy is indispensable for normal trophoblast invasion and fetal-placental development. The advancement of a placental vascular network is significant for the growth and continuance of the developing embryo. Numerous factors are involved in this angiogenic process, involving VEGF (vascular endothelial growth factor), PDGF (platelet-derived growth factor) as well as PAF (platelet-activating factor) [9].VEGF stimulates angiogenesis and augments permeabilization of the blood vessels. VEGF and its receptors are articulated in both the endometrium and in the trophoblastic cells [10].

## 3. ALTERED ADM EXPRESSION ASSOCIATED WITH INTRA UTERINE GROWTH RESTRICTION (IUGR)

Imperfection in the capability of trophectoderm cells to completely infest the maternal uterine wall and modified the vessels which are thought to underlie various serious reproductive conditions [11,12]. It is not unanticipated that changed ADM expression has been associated with numerous of these pregnancy complications. Copious rodent models have provided proof for the requirement of ADM in normal fetal growth. However Yallampalli's laboratory established that antagonism of ADM during pregnancy caused intrauterine growth restriction (IUGR) as well as abnormal placental vascularization with augmented fetal resorption, in the rat [13,14]. The comparable studies in the mouse have exposed that Adm+/− mothers have a high rate of fetal growth restriction, which occurs in all fetal genotypes. The incidence of fetal growth restriction was highest among Adm−/− embryos, stipulating that both maternal and fetal ADM presence during pregnancy may add to normal fetal growth [15]. The conclusions from human studies have not been as constant as animal models. The support on animal studies, evinced probability that altered ADM levels may confer to either the development of IUGR or the effecting adaptive compensation to other primary reasons of IUGR. Yet, the inconsistencies between studies in the human population points to the obligatory of further studies onto determine with certainty how modification of ADM levels might be involved in the pathogenesis of growth restricted pregnancies (Shown in Fig. 1).

### 3.1 ADM in Uteroimplantation Growth

In our present study, we assessed the role of ADM in the instruction of uteroimplantation growth throughout pregnancy. There are a multiple factors like genetic, physiologic, and environmental factors which must all work together as in perfect harmony during pregnancy to produce the so-called "miracle" that is a healthy, full-term baby [17]. Any alteration in this

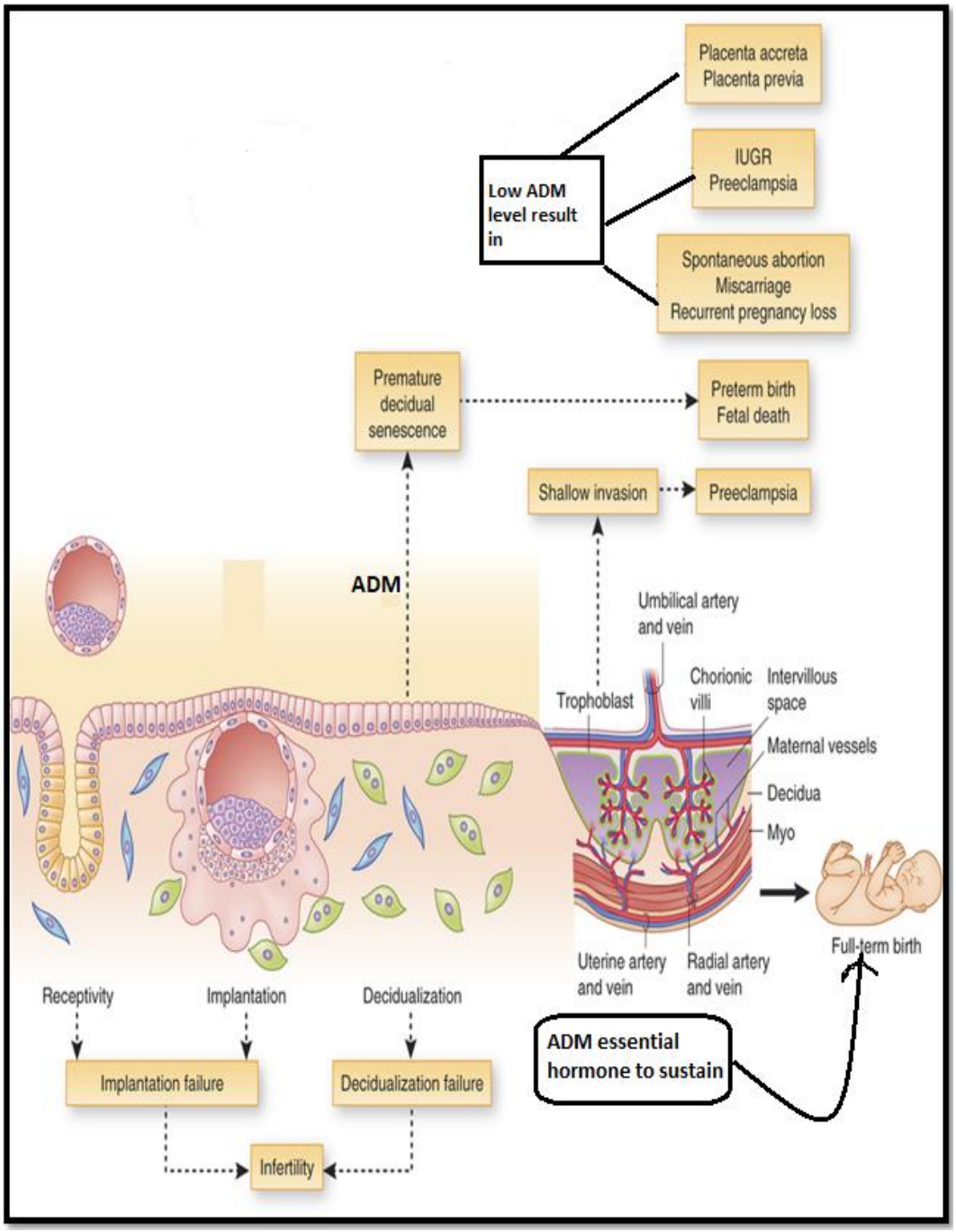


**Fig. 1. Defective receptivity, implantation, and/or decidualization can lead to infertility [16]**

process might result in several pregnancy complications, which includes implantation failure, miscarriage, fetal growth restriction, gestational diabetes, preeclampsia (PE) and preterm birth. Hence there is currently need for vital attention and endeavour in the field for expanding our comprehension of the factors that relates healthy versus unhealthy pregnancies. To illustrate how an environmental modification affects during the course of early pregnancy, several experiments for infusion an antagonist of $ADM_{22–52}$, through osmotic minipumps was beginned on gestational day 2 in rats. These animals received either 125 or 250 µg rat/day of

$AM_{22–52}$ or vehicle only and were sacrificed on day 9 of gestation to assess uterus and implant weights as (shown in Table 1 & Fig. 2).

### 3.2 ADM in Placentation and Maternal-fetal Circulation

One of the main maternal responses to pregnancy is the vascular modification of uterine spiral arteries, which ensures that adequate blood flows to the developing fetus and here the development of the placenta plays a central role to this process. The primary stages of placental development in humans and rodents occur during the process of implantation, when trophectoderm cells from the blastocyst binds and infest into the wall of the receptive uterine endometrium. These trophectoderm cells differentiate into multinucleate trophoblast cells termed extravillous cytotrophoblasts in humans and giant trophoblast cells in rats and mice, which infest the uterine lining and ascertain the vascular connection between fetal placental tissue and the maternal blood supply [18]. High ADM expression is existing in the trophectoderm cells and continues in trophoblast giant cells in the mouse [19]. Although ADM expression in the extravillous cytotrophoblast pedigree has been exposed in the normal term placenta in humans (Shown in Fig. 3c).

**Table 1. Uterus and implant weights of rats killed on gestational day 9**

| Treatment group | Pregnant rats per group (n) | Uterus weight (g) | Implantation wt (g) |
|---|---|---|---|
| Control | 04 | 15.1±0.3 | 1.6±0.1 |
| 125μg$AM_{22-52}$ | 04 | 12.5±0.1 | 1.2±0.06 |
| 250μg$AM_{22-52}$ | 04 | 8.15±0.2 | 0.8±0.03 |

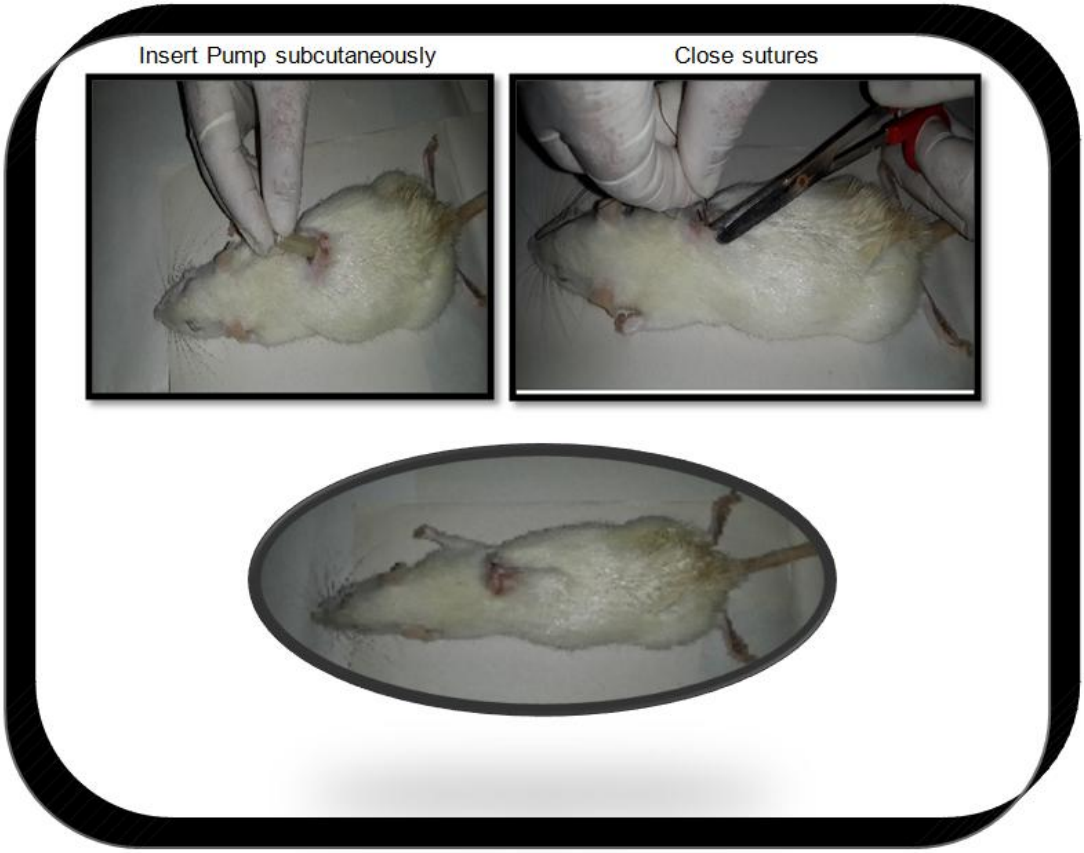


**Fig. 2. Infusion of Osmotic (Alzet) mini pumps**

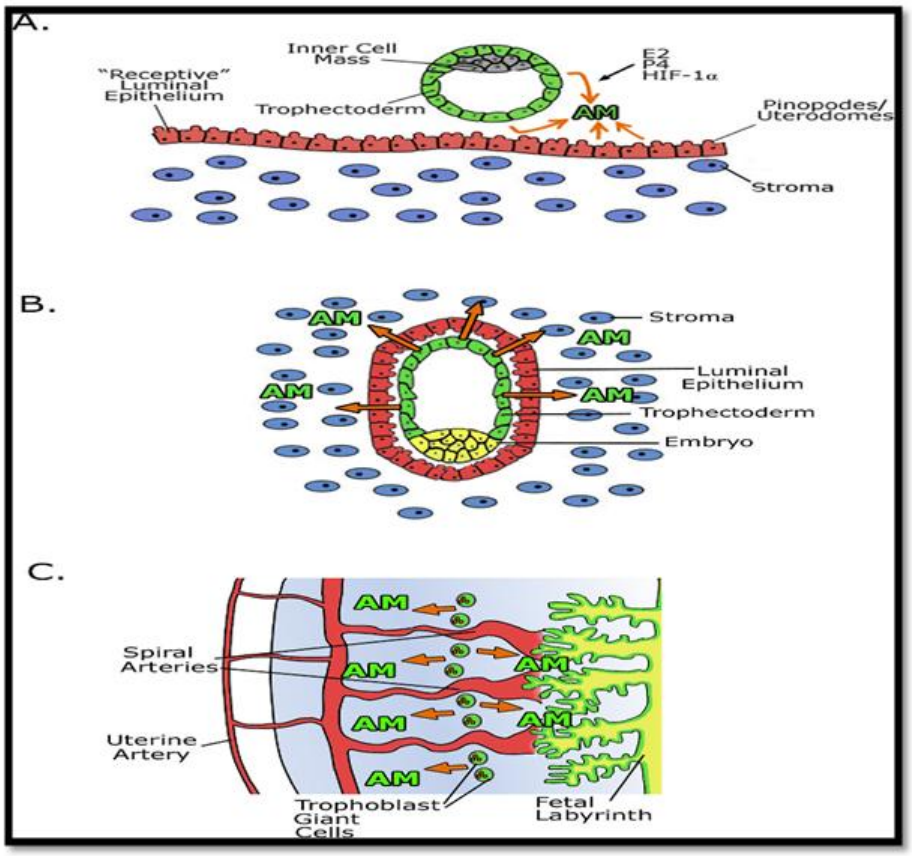


**Fig. 3(A, B &C). Adrenomedullin expression in multiple stages of pregnancy**

*A) AM expression is regulated by estrogen (E2), progesterone, and hypoxia inducible factor 1α (HIF-1a). At the pre-implantation stage, AM is expressed by both the trophectoderm cells and the luminal epithelium, promoting uterine receptivity. The maternal components are colored blue and red while the fetal components are depicted in green and gray. B) At the site of implantation, AM continues to be expressed from the trophectoderm cells and from the luminal epithelium, which is important for successful implantation. C) In the developed placenta, AM is most highly expressed by trophoblast giant cells (mice) or extravillous cytotrophoblasts (humans) and may contribute to the maintenance of placental vascular tone. For both B and C, maternal components are colored blue and red and the fetal components are depicted in green and yellow*

### 3.3 ADM in Implantation

An important role of AM in fertility and implantation has come from well-characterized animal models. Recently several reports including the findings by Padma et al., (2017) have implicated AM in even the earliest stages of pregnancy. Padma et. al., (2017) showed that in a rat model, after injection of BrDU incorporation in rat tail vein, fetal resorption sites are clearly visible. AM expression increases during implantation which has been demonstrated by infusion of ADM antagonist which blocks ADM and results in hypoxic conditions reducing oxygen and blood flow. In the recently carried work, As shown in (Fig. 4). Naturally foetal resorption occurs, when physiologic and environmental factors varied which resulted in resorption sites in early pregnancy.

The control group, $AM_{22-52}$ antagonist of 125 µg/rat/day and 250 µg/rat/day were continuously infused from day 2 of gestation till gestation day8. The ADM antagonist treated group showed reduced uteroimplantation growth and arrow indicates resorption sites. Since BrDU labelling dye is injected, the implanted sites were observed with blue/purple spots in colour and were classified as early implantation. Figure-A is Control; Figure-B is $AM_{22-52}$ treated of 125 µl/rat/day and Figure-C is 250 µg/rat/day of $AM_{22-52}$ antagonist where arrow indicates fetal

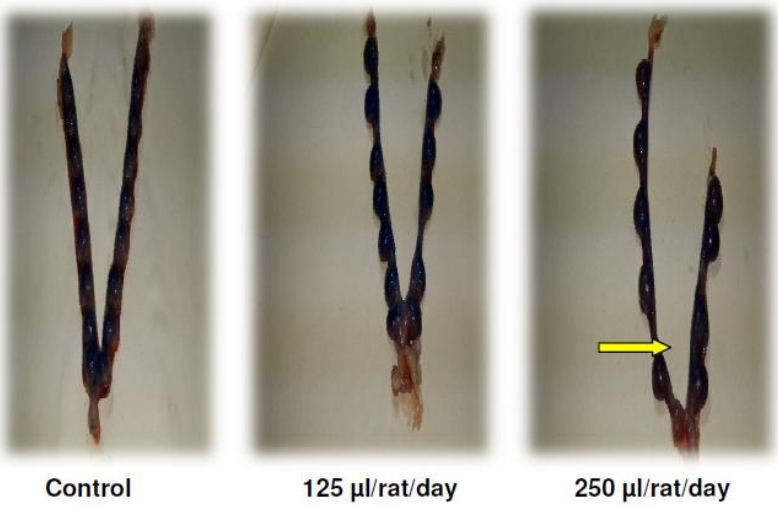


**Fig. 4. Effect of BrDU dye incoorporation during implantation growth**

resorption sites. In the 250 µg/rat/day of $AM_{22-52}$ treated group, on the day 9, the time of conceptuses was clearly visible by small swellings in the uterine wall [20].

### 3.4 Role of ADM in Trophoblast Invasion

The continuity for the role of ADM in trophoblast invasion has come from in vitro studies [21]. It has been exemplified that ADM causes proliferation and invasion in JAr cells, a choriocarcinoma cell line, in HTR-8/SV neo cells and a first-trimester cytotrophoblast ACH-3P cell line. ADM infusion provokes a dose-dependent vasodilation, implying that ADM may help to sustain low placental vascular resistance [22]. Recently, [23] initiated that ADM treatment in rats induces relaxation of the uterine artery and this effect is augmented in pregnancy or with estradiol treatment, providing additional evidence for a functional role for ADM in sustaining vascular tone in pregnancy. In women with unexplained continual pregnancy loss, high plasma ADM was associated with increased uterine artery pulsating index which leads to stress and ultimately leads to miscarriages, from which the authors indicated that increased ADM may be acting in a compensatory role.

## 4. CONCLUSION

The specific spatio-temporal expression pattern of ADM and its receptors is suggestive of its important roles during normal pregnancy, which include embryogenesis, fetoplacental circulation and uterine contraction throughout pregnancy, and implantation during early pregnancy. The antagonism of ADM function *in vivo* has provided strong evidence that ADM function during the pre-implantation period is essential for normal pregnancy. ADM receptor depending on the co-expression of one or more types of RAMP. RAMP1 coupled with CALCRL gives rise to a CALCA receptor, The combination of RAMP2 or RAMP3 results in an ADM receptor. The specificity of CALCRL to CALCA or ADM is therefore modulated by the types of RAMP being expressed at the plasma membrane. Our results revealed that RAMP1, RAMP2, and RAMP3 are expressed in the uteroimplantation regions, suggesting that ADM and CALCA receptors are present in this tissue. Lastly, as for the reason that CLR/RAMP receptor signaling is indispensable for normal transportation of oxygen and nutrients from the mother to the fetus, and since fetal growth retardation from restricted blood flow affects millions of pregnancy each year, these insights may eventually lead to new ways of treating IUGR and preeclampsia using therapeutics that target the CLR/RAMP receptor signaling pathway.

## CONSENT

It is not applicable.

## ETHICS APPROVAL

Animal studies were performed as per institute animal ethics committee regulations and approved by the committee (Reg. No. 1677/PO/a/12/CPCSEA/SPMVV-IEC/2014).

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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