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Review

Establishing life is a calcium-dependent TRiP: Transient receptor potential channels in reproduction☆

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ABSTRACT

Calcium plays a key role in many different steps of the reproduction process, from germ cell maturation to placental development. However, the exact function and regulation of calcium throughout subsequent reproductive events remains rather enigmatic. Successful pregnancy requires the establishment of a complex dialogue between the implanting embryo and the endometrium. On the one hand, endometrial cell will undergo massive changes to support an implanting embryo, including stromal cell decidualization. On the other hand, trophoblast cells from the trophectoderm surrounding the inner cell mass will differentiate and acquire new functions such as hormone secretion, invasion and migration. The need for calcium in the different gestational processes implicates the presence of specialized ion channels to regulate calcium homeostasis. The superfamily of transient receptor potential (TRP) channels is a class of calcium permeable ion channels that is involved in the transformation of extracellular stimuli into the influx of calcium, inducing and coordinating underlying signaling pathways. Although the necessity of calcium throughout reproduction cannot be negated, the expression and functionality of TRP channels throughout gestation remains elusive. This review provides an overview of the current evidence regarding the expression and function of TRP channels in reproduction.

1. Introduction

For a few decades, it has been known that fertilization-induced increased intracellular calcium levels are a '*conditio sine qua non*' in the dialogue between spermatozoa and the oocyte in order to orchestrate the development of new life. However, the function of calcium and its regulation throughout subsequent reproductive events like implantation and placental development remains rather enigmatic. After fertilization and excessive cell division, the blastocyst will travel through the fallopian tubes and arrives in the uterus where it will implant in the endometrium. To allow for implantation, the latter will be appropriately prepared by the combined actions of estrogen and progesterone, culminating during the window of implantation. The transition of the endometrium into a receptive state is accompanied by changes in cell morphology, gene expression and upregulation of adhesion molecules [1]. Successful nidation of the embryo in the endometrium is followed by invasion of trophoblast cells through the epithelium and into the stroma, propagating stromal decidualization in humans or inducing it in rodents [2,3]. Decidualization is the progesterone-dependent differentiation of fibroblast-like endometrial stromal cells into large, secreting decidual cells. The decidua will provide nutrition for the developing embryo prior to placentation. During placental development, trophoblast cells will acquire specialized abilities within the range of migration, secretion of hormones and cytokines, and vascular remodeling. The resulting placenta functions as a surrogate for different organs as it combines a multitude of functions that are separated in the adult. Moreover, it establishes an interface between maternal and fetal circulation in order for gases and nutrients to exchange without evoking an immune response. In addition, the placenta will transport electrolytes like calcium and magnesium to the growing fetus. The transport of these minerals across the placenta was extensively studied and was previously reviewed [4–7]. Concisely, the transport of magnesium and calcium across the placenta increases exponentially towards term to cope with increasing demands and requires the presence of specialized transporters. However, as in all other cells, the proper functioning of placental trophoblast cells as such depends on the intracellular levels of ions, and more specifically calcium [8,9].

Calcium is the most pervasive signaling molecule as it acts as

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secondary messenger and regulates many cellular processes like gene transcription. In normal resting conditions, the free intracellular calcium concentration is below 100 nM, however, it can transiently increase to locally reach the micro molar range [10]. In addition, many signaling events occur below the micro molar range and spatiotemporal concentrations must therefore be tightly regulated. These subcellular concentrations are not regulated by creation or destruction of calcium ions, but rather by the in- and efflux trough calcium transport systems, like calcium permeable ion channels, calcium pumps, calcium binding proteins and calcium exchange molecules [11]. Ion channels can detect a multitude of signals, transducing them into cellular responses, and are, therefore, of paramount importance in many physiological processes. Transient Receptor Potential (TRP) channels are a diverse superfamily of ion channels that act as cellular sensors in order to regulate intracellular calcium and magnesium concentrations [12]. Through the last years, the knowledge regarding the expression and functionality of TRP channels in the reproduction process is upcoming. However, fundamental research on the role of TRP channels in implantation and placentation is still in its infancy and their importance is often disregarded. Not only are ions such as calcium and magnesium important for the growing fetus, they could also play a role as secondary messengers to confer the many functions of trophoblast cells, including cell migration, invasion, hormone secretion, glycogenesis, and vascular remodeling [13]. Placentopathies of unknown etiology are thought to be major contributors to common pregnancy complications such as unexplained miscarriage, recurrent pregnancy loss, gestational diabetes, intra-uterine growth restriction, and preeclampsia [14–16]. Therefore, a thorough understanding of placentation is necessary. This review focusses on the role of calcium during reproduction and summarizes the current knowledge on the involvement of TRP channels during different steps of the reproduction process.

2. The role of calcium in reproduction

2.1. The reproduction processes

During the reproductive cycle, variations in the hormones estrogen and progesterone engender molecular and morphological changes in the ovaries and in the uterine wall. The endometrium, the inner lining of the uterus, is a highly regenerative tissue that consists of two epithelial cell populations, *i.e.* the luminal epithelium lining the lumen of the uterus, and the glandular epithelium that covers the uterine glands, and the endometrial stroma [17,18]. Estrogen-induced endometrial proliferation during the first halve of the reproductive cycle is followed by post-ovulatory progesterone-dependent differentiation, a process called decidualization, which is a prerequisite for successful embryo implantation. Embryo implantation is a multi-step process initiated by apposition of the hatched blastocyst and followed by adhesion, attachment and subsequent invasion of trophoblast cells through the epithelium and into the stroma, propagating stromal decidualization in humans or inducing it in rodents [19]. This maternal response to the implanting embryo is required to overcome the peri-implantation period during which there is no direct contact with the maternal circulation. Decidualization is the progesterone-dependent process of endometrial remodeling and includes the differentiation of stromal cells into enlarged, round, pseudo-epithelial decidual cells. Decidual cells are characterized by the cytoplasmatic accumulation of glycogen and lipid droplets as a source of nutrition for the developing embryo [20]. Hence, before formation of the definitive placenta and the commencement of hemotrophic nutrient supply, *i.e.* the stage of development when fetal nutrition involves the direct uptake of nutrients by fetal placental cells from circulating maternal blood, histotrophic nutrient supply through the decidua is the main source of nutrition.

2.2. The hemochorial placenta

The state of the art concerning placental development in human is limited, with major gaps in knowledge extending from early implantation at day 6–7 to 5–6 weeks of gestation. However, during the last decade, great efforts have been made to improve the understanding of early implantation process. After initial attachment to the epithelial layer, trophoblast cells will differentiate in an outer primitive syncytium, which penetrates deep into the decidualizing stroma by the secretion of lytic enzymes, and an inner cytotrophoblast layer [8,21]. Maternal blood filled lacunae within the syncytium appear and will eventually become the intervillous space. In a second phase of the placental development, the underlying cytotrophoblast layer will penetrate the outer syncytium, giving rise to the primary villi. By the 4th week of gestation, tertiary chorionic villi are fully developed and are surrounded by two trophoblast layers (syncytiotrophoblasts and mononuclear cytotrophoblasts), filled by mesenchyme and fetal blood vessels. Cytotrophoblast cells continue proliferation to form column extravillous trophoblast cells and the cytotrophoblastic shell. At the top of the anchoring villi, the shift from a low to high oxygen environment will induce the differentiation of the column cytotrophoblasts of the villi into invasive extravillous trophoblasts (EVT) and cross the decidual border [22]. *Via* processes known as interstitial and endovascular invasion, the EVTs will invade maternal spiral arterioles or replace the resident endothelial and smooth muscle cells, respectively, resulting in low-resistance conduits. By 10–12 weeks of gestation, the feto-maternal exchange is fully established (Fig. 1).

Hitherto, fundamental research on placentation in human is limited because of ethical and practical considerations. This has incited the use of rodent models, as they share the placental characteristics of being chorio-allantoic with a discoidal shape and a hemochorial interface, where maternal blood is in direct contact with fetal-derived trophoblasts [23]. Murine placentation starts when mural trophectoderm cells evolve to primary trophoblast giant cells (TGC) and invade the mesometrial side of the endometrium. Meanwhile, continuous proliferation of polar trophectoderm cells at the antimesometrial side gives rise to the extraembryonic ectoderm and the ectoplacental cone. The former will expand to form the chorionic epithelium. At E8.5, the mesoderm-derived allantois will cover the chorion in a process called chorioallantoic fusion, which is followed by branching morphogenesis until the dense structure of the labyrinth is formed. Simultaneously, a layer containing spongiotrophoblast cells that originates from the ectoplacental cone resides between the labyrinth and the maternal decidua, and is demarcated by the outer secondary TGC. From E13.5 onwards, a new population of glycogen trophoblast cells will appear in the junctional zone and increase in number by 250-fold at E15.5, while progressively invading the decidua. Eventually, the mature placenta consists of three layers *i.e.* the maternal decidua, the junctional zone, and the labyrinth (Fig. 2) [16].

2.3. Role of calcium during early reproduction events

A. Oocyte maturation & fertilization

During fetal development, > 7 million oogonia have developed into primary oocytes. However, at birth, the oocytes are held in meiotic arrest (prophase I) until puberty, during which a small population will be triggered to mature each cycle. Growth factors released by the granulosa cells surrounding the oocyte upon high levels of luteinizing hormone (LH) will promote oocyte maturation [24]. Although the exact role of calcium in oocyte maturation has been subject to controversy, calcium deprived oocytes will not complete meiosis I properly. In addition, injection of calcium is a necessary and sufficient condition for meiosis resumption *in vitro* [25]. L-Type calcium channels were shown to be involved in the calcium influx that precedes nuclear maturation [26]. Moreover, the achievement of critical hallmarks that endow the