MAMMALIAN OVIDUCTAL EPITHELIAL CELL CULTURE – FUNCTIONAL AND METHODOLOGICAL ASPECTS

HODOWLA PIERWOTNA KOMÓREK NABŁONKOWYCH JAJOWODU U SSAKÓW – ASPEKTY FUNKCJONALNE I METODOLOGICZNE

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Summary: The mammalian reproductive process is regulated on both the molecular and biochemical level by the induction of metabolic pathways crucial for folliculogenesis and oogenesis. Additionally, available data exists describing the molecular basis of ovulation and fertilization. Although the mechanisms responsible for normal embryo growth and development in preimplantation stage are well defined, there is not a wealth of data describing the interaction between the embryo and the oviduct. Therefore, the development of new methodology for oviductal epithelial cells (OECs) and endometrial epithelium culturing is of interest, primarily focusing on interactions between the developed embryo and oviductal epithelial cells as well as the fetus and endometrium. In this review, we present data examining the molecular basis of ovulation and fertilization, as well as differentiation of OECs. The interactive mechanisms between OECs and embryos post-fertilization and subsequent embryo growth are also described. Furthermore, the most current data regarding species dependent methods of OECs *in vitro* culture are discussed.

Key words: oviducts, embryo implantation, ovum transport

Streszczenie: Właściwy przebieg procesów związanych z rozrodem jest u ssaków regulowany na poziomie molekularnym i biochemicznym poprzez indukcję szlaków metabolicznych kluczowych dla folikulogenezy i oogenezy. Ponadto w literaturze dostępne są dane odnoszące się do molekularnych podstaw procesów owulacji i zapłodnienia. Mechanizmy odpowiedzialne za prawidłowy rozwój zarodka w stadium przedimplantacyjnym zostały dobrze poznane, nadal jednak mało jest badań odnoszących się do interakcji pomiędzy zarodkiem a komórkami nabłonka jajowodu (OECs). Dlatego też rozwój

metod hodowli pierwotnej komórek nabłonka jajowodu (OECs) oraz komórek błony śluzowej macicy jest interesującym zagadnieniem, głównie z uwagi na interakcje pomiędzy matką a rozwijającym się zarodkiem a następnie błoną śluzową macicy a płodem. W artykule przedstawiono dane odnoszące się do molekularnych podstaw owulacji i zapłodnienia, jak i różnicowania się komórek OECs. W przedstawionej publikacji omówiono także zagadnienia odnoszące się do interakcji pomiędzy komórkami OECs i zarodkami poprzedzających zapłodnienie i późniejszy rozwój zarodka. Ponadto, przedstawiono zależne od gatunku różne metody hodowli *in vitro* komórek OECs.

Słowa kluczowe: jajowody, implantacja zarodka, transport komórki jajowej

INTRODUCTION

Successful mammalian embryo implantation and subsequent fetal growth and development are a complex process that requires activation of several biochemical and molecular mechanisms. Ultimately, these mechanisms lead to the induction of crucial metabolic pathways involved in pregnancy progression and maternal- fetal interaction. The entire pregnancy outcome process can be divided into three crucial steps: (1) formation of the antral follicle and further ovulation, (2) successful monospermic fertilization, and (3) implantation of embryos in the prepared endometrium. However, these simple processes are coordinated and regulated by a specific and highly specialized mechanism requiring proper cellular interaction [12].

First, the sperm-egg interaction is regulated by expression of fusion proteins and receptors such as zona pellucida proteins (ZPs) and integrins [17, 19]. Activation of all of these proteins is responsible for gamete recognition and fusion. After fertilization, the developed embryo interacts with oviductal epithelial cells (OEC) and reaches the uterus. Next, the growing embryo arrives at the proper site of the endometrium, which is ready for implantation and decidualization. In the literature, there is inadequate data describing the compound process of embryo-oviductal epithelial cell interaction. Moreover, the methods of isolation and culturing of these cells are still not entirely known. Therefore, the goal of this review is to highlight the process of this interaction, describe the physiological association between the growing embryo and OEC, and reveal several methodological aspects of OEC primary culturing.

BIOCHEMICAL AND MOLECULAR ASPECTS OF OVULATION AND FERTILIZATION

Ovulation is the process that initiates every reproductive cycle of the mammalian female. Successful ovulation is a complex process whereby ovarian follicles reactivate oocyte meiosis, creating a rupture pore in the apical follicle wall, and

initiate tissue restructuring and differentiation to form the corpus luteum. The surge of two gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), are released from the pituitary. This then triggers ovulation and is a crucial prerequisite for fertilization and embryonic development [15]. In the preovulatory follicles, a cascade of proteolytic enzymes including plasminogen activator (PA) and plasmin and matrix metalloproteinases (MMPs) is induced by the LH surge. The release of these enzymes starts the degradation of the perifollicular matrix and a meshwork of collagen fibers, which strengthens the follicular wall [30, 34]. Plasminogen activator inhibitor-1 (PAI-1) and tissue inhibitor of metalloproteases-1 (TIMP-1) are enzymes that control increased ovarian proteolytic activity [10]. Increased expression of these two specific proteinase inhibitors within the theca of growing follicles secures their development by protecting them from enzymes diffusing from ovulatory follicles. The gonadotropin surge, which stimulates ovulation, increases follicular blood flow, vascular permeability, and follicular volume [4, 13]. These changes are triggered directly by released gonadotropins or by compounds produced as an effect of gonadotropin stimulation. Factors and local mediators expressed as a result of the LH/FSH surge ensure coordination of these two cascades, resulting in the tear of the follicle wall [28].

Following successful ovulation, fertilization is the next step, finally leading to embryo implantation and pregnancy. Fertilization is the process by which eggs and spermatozoa interact, achieve mutual recognition, and interact to create a zygote, which then develops to form a new organism [1, 16]. The entire fertilization process is divided into five steps. First, the sperm must bind in a species-specific manner to the thick extracellular coat, or zona pellucida (ZP), of the egg [33]. While bound to the egg, spermatozoa have to undergo the acrosome reaction, or cellular exocytosis [2]. Next, sperm penetrates the extracellular coat [6]. When the spermatozoon reaches the perivitelline space between the plasma membrane and the egg ZP, sperm then binds to the plasma membrane and fuses with it [31]. The fusion with a single spermatozoid prevents the egg plasma membrane from fusing with other sperm that have penetrated the ZP. At this point, the egg has been fertilized and becomes a zygote, and free-swimming sperm are no longer able to bind to the ZP [11, 18].

INTERACTIONS BETWEEN EMBRYO AND OEC

The oviduct is an essential organ in reproductive biology. It supports gamete transport, maturation, capacitation, fertilization, early embryonic growth, and embryo transport to the uterus [8, 21, 20]. Oviduct fluid consists of a serum filtrate, follicular fluid, and oviduct-specific secretory products from secretory cells

[22]. During the follicular phase, after the oestrogen and LH surge, secretions from oviduct secretory cells increase [1]. Moreover, oviduct proteins interact with gametes and improve the efficiency of *in vitro* fertilization (IVF) in porcine [14], bovine [23], and human [29] species. Some proteins involved in this process have been identified: osteopontin in bovine and porcine [14, 25], and atrial natriuretic peptide A (ANPA) [3, 35, 36], in bovine, porcine and human [23, 7, 24]. Bovine OEC cultured *in vitro* produce and secrete soluble factors which assist embryonic development [10, 27]. In 1994, Nancarrow and Hill reported that oviduct-specific glycoprotein influence *in vitro* development of embryos in sheep [27]. In addition to the secretion of embryotrophic substances, oviductal cells are able to remove toxic compounds in culture medium that are harmful to embryonic development [5]. Additionally, oviductal epithelial cells may regulate oxygen tension in the immediate vicinity of the embryo. Nagao et al. [26] studied the interaction of oxygen concentration and the presence of BOEC on the development of bovine embryos *in vitro* in matured and fertilized (IVM/IVF) oocytes in a protein-free medium. Their results suggested bovine IVM/IVF embryos can successfully develop to the blastocyst stage without the bovine OEC feeder layer at low concentration of oxygen (5%). Thus, we can assume that one of the crucial functions of OEC during embryonic development may be reducing the oxygen concentration in the embryonic environment.

IN VITRO CULTURING OF MAMMALIAN OEC

The standardized, optimized, and well characterized models of *in vitro* oviduct epithelium cell culture are very important for scientists because of their multiple applications in biomedicine and biotechnology. The OECs are of interest since the molecular mechanisms responsible for maturation and transport of gametes, ovum fertilization, early embryo development, and interactions between OEC, gametes, and embryos must be explored. One of the first OEC cultures was described by Witkowska in 1976 [32]. Witkowska described cultivation of bovine OEC on the cover glass in Parker-199 medium supplemented with 10% calf serum. First, cultures were cultivated for a short time because there was sparse knowledge concerning OEC culturing. Over time, *in vitro* OEC culturing has been improved and culture time has been extended up to six weeks (10-15 passages) [32]. Chen et al. reported that porcine OEC were successfully cultured for six weeks [9]. Their culture system maintained high reproducibility and stable conditions, thus over 95% of cultures achieved a fully differentiated phenotype. Throughout the establishment and standardization of this method, they examined several media types with different supplementation in order to achieve the best effects, highest

reproducibility, and longest cultivation time. Chen et al. analyzed two different media: conditioned (CM) and unconditioned (NM) medium, supplemented with different sera: FBS Gold (CM-G) and FBS Superior (CM-S) [9]. Unconditioned medium consisted of Ham's F12 medium supplemented with 10% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, 50 µg/mL gentamicin, 1 µg/mL amphotericin B, 10 µg/mL reduced glutathione and 10 µg/mL ascorbic acid. Between NM and CM was one difference: CM has been enriched with growth factors obtained from 3T3 Swiss albino embryonic fibroblasts. The 3T3 cells were cultured in Ham's F12 with 10% FBS then the 3T3-enriched medium was diluted in NM medium at 1:2 (v/v) ratio to obtain CM. There was an observed impact of CM on the quality of porcine oviductal epithelial cells (POEC). In comparison to NM, cultures grown in CM were significantly higher than cultures grown in NM. Cells grown in media supplemented with different sera (CM-G and CM-S) achieved a fully differentiated state and showed comparable cellular heights, thus different sera types did not have a significant impact on POEC culture.

POSSIBLE APPLICATIONS OF OEC PRIMARY CULTURE MODELS– FURTHER PERSPECTIVES

Optimized and stable primary culture of OEC could be used widely by scientists in several fields of research. Co-culture of OEC and embryos is a useful tool to improve procedures for *in vitro* embryo production as well as enhancement of our knowledge about fertilization and its control pathways. Co-culture of OEC and spermatozoa allows a great opportunity to study mechanisms of the acrosome reaction, capacitation and fertilization, The bovine spermatozoa co-cultured with bovine oviductal epithelial cells (BOEC) exhibited changes in capacitation and maintained motility and fertilizing capacity for a long time period. It is believed that OECs synthesize and secrete different proteins, glycoproteins, and growth factors. Further research on OEC secretory products may enhance our knowledge of these product functions and describe their role in reproductive processes. Furthermore, long-term culture of OEC gives us the ability to simulate the whole oestrus cycle *in vitro*. Long-lasting OEC culture could also lead to the investigation of toxic effects of any compound on the oviduct epithelium.

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