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IMPACTS OF CULTURE MEDIA AND PROSTACYCLIN ON EMBRYO DEVELOPMENT *IN VITRO*: A SYSTEMATIC REVIEW

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ABSTRACT Previous research has suggested the inclusion of prostacyclin (PGI2) in culture media improves embryo quality and pregnancy outcomes. PGI2 is found naturally in large quantities in the oviducts during fertilization and early stage embryo development, and could potentially be beneficial for fertilization and early embryo growth. It has been theorized that PGI2 stimulates the an anti-apoptotic pathway in early embryonic stages, and can prevent embryos from going through apoptosis, or programmed cell death. Developing embryos often suffer from apoptosis while in culture for ART procedures, which could potentially be halted by inclusion of PGI2 in the growth media. If a PGI2 media additive could provide some stability to the development of the embryos during IVF, it is possible the resulting healthier cohort of embryos could reduce the number of IVF and embryo transfers that patients must undergo. It has recently been reported that Iloprost, a biosynthetic PGI2 analogue and cardiovascular active drug, enhances embryo development and prevents apoptosis. It has been hypothesized that the mechanism involves the induction of the survivin pathway. This review highlights culture media influence on embryo development, the importance of PGI2 and prostaglandins in embryo development, the challenges embryos face during development, and how these are connected.

KEYWORDS : Embryo development, Prostaglandin, Prostacyclin, Blastocyst, Embryo culture media, Apoptosis

BACKGROUND

Culture media conditions have changed through the years as research has been conducted to determine the most optimal culture media possible for embryo development. Initially, culture media started out as relatively simple solutions with salts and various amino acids. Researchers became more complex over time, with some media containing nearly eighty different components [1]. In the early 1990's, the development of co-culture systems proved to be an improvement over the use of Earle's balanced salt solution [2]. Yeung found that there was a significant increase in the percentage of blastocysts that hatched from the zona pellucida when utilizing a co-culture system including human oviductal cells [2]. A later study supported this theory by demonstrating that inclusion of human oviductal cells reduced apoptosis in developing embryos in an in vitro culture environment [3]. Researchers were able to demonstrate that embryos co-cultured in human oviductal cells underwent significantly less apoptosis than those that developed in culture media alone [3, 4]. Researchers utilized a co-culture system to develop bovine embryos in vitro and concluded that supplementation with human or bovine oviductal cells increased the success rate of embryo development significantly [4]. Researchers then stated that amino acids would be "prudent in the inclusion in media" due to the amino acids that are available as nutrients for the developing embryo [5].

Over time, commercial groups continued searching for the most successful culture media condition. Due to the numerous variations of culture medias, a meta-analysis of birthweight data from over 1600 births from IVF patients was conducted and found that the type of media used during the early developmental stages doesn't have a statistical effect on the birthweight or length of the offspring [6]. Lin analyzed the data from offspring developed in Global Media (LifeGlobal; Guilford, CT), G5 Media (Vitrolife; Gottenburg, Sweden) and Quinn's Advantage Media (Sage; CA, USA), and found that there was no media associated with higher birth weights or lengths. Another group of researchers compared Global media and Quinn's Advantage Cleavage media (Sage; CA, USA) and found no significant difference between the developmental success rates of either group of embryos [7]. However, Sunde found that embryos in a low nutrient environment become "thrifty" and conserve nutrients until transferred into the recipient, at which time they tend to over-compensate and overgrow [1]. However, according to Sunde *et al*, there is no discernable or statistically significant difference between culture media and their effects on embryo development that sets one culture media above the rest [1].

After almost forty years of ARTs, researchers are still investigating which nutrients, proteins and supplements should be added to an in vitro culture environment to induce optimal growth [7, 1]. Development of embryos *in vitro* is very

different from the development of embryos *in vivo*. In the maternal environment, embryos are constantly manipulated and transferred through the oviduct and then the uterus before implanting into the lining of the uterus [8]. This mechanical stimulation could be beneficial for embryo growth and development [8]. In most *in vitro* environments, the embryo is in a stable, secure location that doesn't allow for much mechanical stimulation of the embryo [8]. The quality of the culture medium can also influence the developmental progress of the embryo, and can cause regression and death of the embryo [9].

With numerous differences between the two developmental environments, many similarities remain. Culture media utilized in the *in vitro* environment has been constantly changing to closely mimic the perceived environment of the *in vivo* system and to satisfy the nutrient requirements of embryos during early stages of development [9]. Some embryology programs also utilize small, electrically powered, chip technology that induces mechanical stimulation of the embryo during those early stages of development [8]. Lastly, it is the ultimate goal of embryologists to introduce the embryo back into the womb of the maternal environment at the necessary time-point to ensure implantation of the blastocyst and continued development of the embryo [10].

Cell Death in Embryo Development

While IVF and other ARTs have become routine, there are still issues which limit success these would include, but are not limited to: ovulatory dysfunction, sperm abnormalities, low sperm count, low motility, and blockage of the oviduct. Embryologists also have the difficult task of deciding how many embryos to transfer during an IVF/embryo transfer procedure. Typically, embryologists are transferring between 2-3 embryos per cycle to hopefully attain one healthy baby [10]. However, 10-15% of the time, twins and triplets are a result of multiple embryo transfer (CDC, 2014).

A challenge to successful fertility treatment is embryos spontaneously undergoing apoptosis and dying during development [11]. "Programmed cell death has been observed in mammalian blastocysts obtained in vivo, and in vitro" [12]. Jurisicova further explains that since there are so few types of cells to begin with, "if abnormal cells weren't eliminated, their contribution might have severe consequences for embryonic development and survival." However, uncontrolled apoptosis can lead to embryonic death at any point either during culture or after embryo transfer. Embryonic death after transfer will result in early term abortion and pregnancy loss [11].

Necrosis

Cell death in early embryonic stages is typically the result of either necrosis or apoptosis. Necrosis is the typically "characterized by uncontrolled cell death" [13]. Necrosis differs from apoptosis in that it is not planned, it results in cellular exhaustion, and can be stimulated by physical stress or injury [13]. Cell death and regression is beneficial in certain developmental aspects, such as the regression of the webbing between toes and fingers of humans [14]. However, in the case of necrosis, the cell death is uncontrollable and usually fatal [13].

Due to the inability of the body, or embryo, to regulate necrosis, it is very demanding on the system, can halt maintenance of ion pumps, and induces the immune response [13]. Apoptosis and necrosis do crossover in some instances, with apoptotic cells that don't completely undergo apoptosis typically undergoing necrosis [15]. Necrosis is also similar in some instances to apoptosis, with the body inducing programmed necrosis at times [15]. When the body induces necrosis, the cells will undergo "cytoplasmic granulation" and "cellular leakage" [15]. The necrosis pathway has also been described by Vanden Berghe *et al* to be initiated by Tumor Necrosis Factor (TNFR) 1. This contrasts the theory that necrosis is "accidental" or stress-induced cell death [16].

Necrosis, overall, is typically initiated by physical stressors and/or cell injury, and leads to an uncontrollable inflammation response that cascades until the cell dies [16]. By causing an uncorrectable shift in cell homeostasis, the stress or injury causes the body to recognize that the cell is beyond repair and initiates an immune-mediated response that becomes fatal for the cells involved [16]. However, even with the differences between apoptosis and necrosis, they are very tightly intertwined and utilize either pathway in different circumstances [16, 15].

Apoptosis

Many types of cancers survive because they have over ridden apoptosis, theoretically through the use of survivin [17]. In a study on inducing apoptosis, researchers found that treatment with staurosporine (STS) and/or cycloheximide (CHX) led to programmed cell death in all nucleated cells that were treated [18]. However, their research showed that blastomeres in the 2-4 cell stage seemed to be unusually resistant to the chemical compounds with only limited apoptosis [18]. Although these early developmental cell types were resistant to the apoptosis-inducing chemicals, blastocysts weren't as resistant [18]. When treated with STS and CHX for 26-60 hours, more than 90% of the cells died, demonstrating that blastomeres become more sensitive to apoptosis after differentiation [18].

Research has shown that there are several potential genes in the body that act as "killer genes" that induce apoptosis [12]. Activation of the Ced-3/Ced-4 genes seem to initiate apoptosis, whereas the Ced-9 gene seems to suppress the cell death tendencies that the first two genes seem to induce [12]. In addition to these normal genetic pathways programmed to initiate apoptosis, there are several chemicals used by researchers to induce apoptosis in experimental settings. One such chemical is YM-155, a "novel survivin suppressant", that halts survivin from being active through suppression of survivin promoter activity [17].

Prostaglandin Biochemical Pathway

Prostacyclin, prostaglandins, and their associated pathways have numerous effects on cell function [19]. Cyclooxygenase (COX) is the rate-limiting enzyme in the prostaglandin biosynthetic pathway and is largely responsible for producing the prostaglandins, including PGI2. The COX enzymes are responsible for the conversion of arachidonic acid into PGH2, which is a substrate for every other form of prostaglandin [20, 21]. Peroxisome proliferator-activated receptors (PPARs), specifically PPARδ, are receptors that mediate blastocyst implantation through the use of COX-2-derived PGI2 [22]. PPARδ is a receptor that helps stimulate the pathway from arachidonic acid to PGI2 [22]. COX enzymes exist as both COX1 and COX2 [23]. COX2 is the rate-limiting enzyme that is responsible for producing PGI2 [24].

Knockout mice with COX-2 deficiencies exhibit multiple reproductive failures. These include the inability to ovulate, even though the mice exhibit normal follicles; infertility, potentially due to impermeability of the zona pellucida, and inability of the embryo to implant [11]. PGI2 has been demonstrated to enhance mouse embryo hatching from the zona pellucida in experiments with a general COX inhibitor [25]. The initial experiments demonstrated suppression of both COX1 and COX2; in a later experiment, researchers used targeted COX inhibitors and found that COX1 inhibition did nothing to hatching rate as compared with the control [25]. However, COX2 inhibition did show a very comparable decrease in the hatch rate as with the general Cox inhibitor [25]. When using that inhibitor in culture, Huang *et al* (2003)

started adding different prostaglandin analogues to see if they could restore hatching potential, by examining Iloprost, PGE2, and a PGF2δ analog. It was found that Iloprost was the only compound to return the hatching potential to the embryo [25]. In fact, it appeared to enhance hatching as those embryos cultured with Iloprost hatched at higher hatching rate than the control embryos[25].

These findings support the use of Iloprost supplementation in culture media, and further studies have also been conducted to determine if the action of PGI2 is mediated through I-PGI2 receptors (IP) [26]. Researchers found that Iloprost had no effect on, and did not enhance, hatching when the IP receptors were genetically removed from mice [26]. "These findings provide strong evidence that IP receptors play a crucial role in the in vitro development of pre-implantation embryos" [26]. The researchers also concluded that PPARδ was activated through signaling pathways which are self-activated through IP receptors [26]. This is an important finding, as PPARδ is a controlling factor in the pathway that converts arachidonic acid to PGI2 [24, 22]. PGI2 also seems to be a PPARδ ligand that stimulates gene transcription through its formation of a heterodimer with retinoid X receptor [21]. In previous studies, researchers have stimulated expression of the PPARδ - mediated genes, demonstrating that two of these genes, 14-3-3 and phosphoinositide-dependent kinase-1 (PDK-1), have anti-apoptotic actions that help protect cells from apoptosis[26].

Effects of Prostacyclin on Development & Reproduction

It has been shown that mice have an increase in production of ovarian PGI2 2-3 days post copulation; this spike induces about 10 times the normal amount compared to a non-pregnant mouse [24]. After treatment of mouse embryos with COX-2, PGE2 and a COX-2 inhibitor, results indicated that COX-2 is involved in the protection of embryonic stem cells from oxidative apoptosis[27]. Liou *et al* (2007) also showed for the first time that COX-2, PGES, and EP1-3 were expressed in mouse embryonic stem cells [27]. These prostaglandin receptors and proteins prevented oxidative apoptosis through the anti-apoptotic actions of PGE2 and the EP2 receptor [27]. This protection from apoptosis continues to strengthen the argument that certain prostaglandins can have an anti-apoptotic role on certain cells [27, 28, 29]. However, prostaglandins have different effects on different cell types[19].

Whereas PGE2 confers apoptosis resistance to embryonic stem cells and non-small cell lung cancer cells, it tends to mediate apoptosis in T cells, thymocytes, and B cell lymphomas [27]. PGE2 also confers survivin-dependent apoptosis resistance in human dendritic cells [30]. Some prostaglandins, specifically PGF2δ, cause early-term abortions in high concentrations in animals[14, 31, 19]. PGF2δ has a "leuteolytic action" on the female reproductive tract and can cause embryo loss through contractions of smooth muscle, leading to embryo death and ultimately expulsion from the uterus[31, 19]. In the U.S., PGF2δ is a legally accepted method of inducing an early-term abortion in pregnancies up to 3-weeks of development[19].

PGI2 is still being studied for its role in anti-apoptosis[32]. In a study performed in 2002, researchers found that human oviducts synthesize abundant PGI2 [33]. With this realization, researchers found that PGI2 affects oviductal tissue in an inverse relation with PGF2δ. While PGF2δ causes muscle contractions and tremors, and can cause early-term abortions and apoptosis in a high concentration, PGI2 decreases the amplitude of contractions[31, 19, 33]. More research is needed on the differences between the two different forms of prostaglandin and their relationship in various tissues types, especially the reproductive tract. Researchers have found that supplementing culture media with PGI2 enhances the complete hatching from the zona pellucida [25]. Unlike a number of other reproductive hormones, PGI2 doesn't appear to have any effect on sperm motility [25]. However, PGI2 seems to cause a reduction in uterine inflammation and thus leads to easier implantation of the embryo; while other prostaglandins lead to inflammation and a response of the immune system[24].

CONCLUSION

The field of reproduction has advanced by leaps and bounds in a very short time. Further modification of culture media to include factors which promote embryo growth and hatching seem a logical step to improving IVF and embryo transfer success. With the proven success of the inclusion of PGI2 and its effects of COX-2 in the realm of reproduction, a PGI2 analog should lead to better quality embryos leading to increased reproductive success. In conclusion, embryo culture media has advanced rapidly, and the next logical step seems to be the inclusion PGI2 in IVF medias, which would enhance embryo development without compromising sperm function or embryo morphology.

Declarations

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Not applicable.

Consent for publication

Not applicable.

Availability of data and material

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Competing Interests

The authors declare that they have no competing interests.

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Authors' Information

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