HAZARDS AND SHORTFALLS ASSOCIATED WITH ALBUMIN IN EMBRYO CULTURE, HANDLING AND CRYOPRESERVATION MEDIA

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Abstract

Are the embryo culture, handling and cryopreservation media (EM) and their ingredients safe for the patient, the embryo, the offspring and to the healthcare worker in assisted reproduction treatment? The EM is composed of defined and undefined components. The use of chemically defined components in the EM is scientifically sound, but the use of non-uniform components is less scientific. The main undefined component in the EM is human serum albumin (HSA). This discussion attempts to bring to the fore the safety issues or hazards, disadvantages, inappropriateness, non-compliance with norms and incompatibility with cultural values that is associated with the use of non-uniform biological components in EM. Some of these include the potential for transmission of pathogenic agents, undeclared harmful proteins, and contaminant donor miRNAs. The donor miRNAs pose risk of transcriptomic modification, may in turn could compromise the genetic purity of the lineage of the progeny. The latter is not acceptable in many cultures. This investigation assumes that a vast majority of the workers in assisted reproduction are not aware of the consequences of using non-uniform biological components in the EM. In conclusion numerous potential hazardous and non-compliance with norms occur with the use of albumin in EM. In view of the hazards and disadvantages associated with the use of non-uniform albumin in EM it is suggested that efforts are made to utilize synthetic EM in assisted reproduction procedures. .

Disclaimer: All authors declare no conflict of interest whatsoever with the exception of J. Ali who is the inventor of a series of synthetic embryo culture, handling and cryopreservation media.
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Introduction

The embryo culture, handling and cryopreservation media (collectively referred to in this manuscript as embryo media or EM) contain numerous constituents, both defined and undefined. One fundamental question is whether the EM and their ingredients, particular the undefined components, are safe for the IVF patients and the offspring that were generated in such media. Another important question is whether it is safe for medical laboratory professionals who regularly come into contact with such media? Insufficient information and knowledge on the hazards or toxicological properties of the components of the EM used in ART media persists (Sunde et al., 2016). It has not been adequately addressed whether the embryo culture, handling and cryopreservation media are safe for use in humans. Nearly everyone who works in the field of therapeutic assisted reproduction assumes that the EM is safe and utilizes them on a routine basis. Are they really safe?

The defined components in the EM are mainly synthetic components such as carbohydrates, amino acids, minerals salts, energy substrates, metabolites, synthetic hormones and organic chemicals (eg: Glycerol. Ethylene diaminotetra-acetic acid (EDTA), ethylene glycol (EG), dimethyl sulfoxide (DMSO), propylene glycol (PG) or propanediol (PROH), HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), etc. Whereas the undefined components are protein sources such serum, human serum albumin (HSA) or HSA-derived products, growth factors (some growth factors may be synthetically derived but may still contain contaminants unless they are very well purified), hormones of biological origin, protein extracts (example: egg yolk), and any product of biological origin.

Amongst these, the serum proteins or HSA or HSA-derived products were considered essential components for the EM until the successful formulation of synthetic embryo culture and handling media some 20-odd years earlier (Ali, 1997; Ali, 2004; Ali et al, 2000). The present discussion focuses on one specific ingredient of the EM, namely, human serum albumin (HSA) or HSA-derived products that has raised the concern of healthcare workers in assisted reproduction therapeutics (van Os et al. 1991; Kemmann, 1998; Ali et al., 2000; Matson and Tardiff, 2012; Sunde and Balaban 2013; Peirce et al., 2015, 2017, and many more). The HSA or HSA-derived products are biological products and consequently are not defined and its composition non-uniform between batches (Sunde et al., 2013) as they are obtained from different batches of donors. HSA or HSA-derived products are known to contain numerous other undeclared contaminant components that could be harmful (Dyrlund et al., 2014) to the patients, their gametes, embryos, and babies born of IVF embryos.

Indeed, the European Parliament issued the European Union Tissue and Cell Directive in 2004, (Directive 2004/23/EC), urging member states to move away from the use of non-uniform biological components in healthcare products by April 2007 (EU, 2004) however in assisted reproduction treatment the use of HSA or HSA-derived products has persisted to the present times. This communication explores the disadvantages and impact of the use of undefined biological components, in particular, HSA or HSA-derived products in assisted reproduction treatment procedures and their potential and possible detrimental effects to the embryo culture system (van Os et al. 1991; Kemmann, 1998; Ali et al., 2000; Matson and Tardiff, 2012; Sunde and Balaban 2013; Peirce et al., 2015, 2017). In recent years it has come to light that some aberrations occurs during embryo development in vitro which noted that individuals born of IVF treatment are prone higher incidences of some diseases (see reviews by Ahmadi et al., 2023; Berntsen et al., 2019; Hart and Norman, 2013a,b; Kalra and Molinaro, 2008; Corabian and Hailey, 1999) but most of these adverse outcomes are probably not directly related to the use of donor proteins in EM.

The impact of individual media components on gametes, embryos and the long term impact on health of individuals born following ART treatment are not fully understood (Matson and Tardiff, 2012; Peirce et al., 2015, 2017; Sunde et al., 2016) but the incidences of aberrations appears to increase among individual conceived from ART compared natural means (Katari et al., 2009; Young et al., 2001; Farin et al., 2006; Turan et al., 2010; Nelissen et al., 2013; Fidanza et al., 2014; Lazaraviciute et al., 2014; Toschi et al., 2016; Choux et al., 2018; Lou et al., 2019; Mani et al., 2020; Haber and Ptak, 2021; and many more reports) which indicate the culture milieu of embryos could impact the health of the in vitro derived embryo and the progeny resulting therefrom.

Evolution of the EM

From the early attempts to culture embryos in the 1900s (Mark and Long, 1912; Bracket, 1912, 1913; Maximov, 1925; Lewis, Gregory, 1929; Pincus, 1930; Gregory, 1930; Gregory and Castle, 1931; Lewis, 1931; Lewis and Hartman, 1933; Hammond Jr., 1949) to the successful development of embryo culture medium in the mid-1950s (Whitten, 1956; 1957) to the present (Brinster, 1971; Menezo et al., 1984; Loutradis et al., 1992; Lawitts and Biggers, 1993; Quinn, 1994; Gardner et al., 1996; Summers and Biggers, 2003; Quinn, 2004; Roberts, 2005) embryos have always been cultured in vitro in nutritional solutions made completely of chemically defined solutions that are

supplemented with donor serum proteins or "serum replacement" products/derivatives. An extensive account of the evolution of embryo culture medium and the use of biological components in embryo culture media over the last century has been described (see review by Nielsen and Ali, 2010). The main body of the media is chemically defined but when biologically derived components were added to it, they no longer retain their status as chemically defined. The use of chemically defined EM is scientifically sound but not the non-uniform component(s).

These media supplements come from human donors, or include biologically produced ingredients. Previously, albumin from animal donors (Bovine serum albumin or BSA (Loutradis et al., 1992) were utilized in the EM for the culture of human embryos but the use of animal proteins in human EM products has since been discontinued about two decades earlier. It must be borne in mind thousands of viruses and other potentially infectious microorganisms thrive in cows, the preferred animal for serum protein supplements used in cell culture and vaccine media (Rock, 1996) that can jump host to cause much damage to human health. Mad cow disease can be passed from cows to humans (Marwick, 2000) through vaccines, which suggest that it could likewise possibly be transmitted through embryo culture media supplemented with BSA contaminated with BSE prions. Bovine immunodeficiency virus (BIV), similar in genetic structure to HIV, was found in some cows (Miller,2004). The same situations could occur in the human. Blood products used in cell culture media remains a serious problem and a health risk to humans. Protein supplements can be irradiated to destroy viral pathogens but not prions. Even extreme heat treatment may not destroy prions.

Early attempts to culture human embryos in synthetic medium were somewhat compromised because of the need to incorporate proteins in the fertilization medium (Caro and Trounson,1986; Serta et al. 1997; Parinaud et al., 1999) because protein was thought to be essential for sperm capacitation (Aitken et al., 2007; Bailey, 2010; Brewis and Gadella, 2010). An efficacious and completely synthetic protein-free EM that could generate viable human embryos and pregnancies was first communicated by Ali (Ali, 1997) and published in the years 2000 and 2004 (Ali et al., 2000; Ali, 2004) but remains to be made available commercially to the fraternity.

Tangible and potential risks associated with the use of biological components

The risks and or the inappropriateness of utilizing undefined nutrient components in the embryo culture medium have been acknowledged and discussed by previous researchers since the 1960s to the present times (Cholewa and Whitten, 1965; Caro and Trounson, 1986; Li et al., 1996; Schramm and Bavister, 1996; Serta et al. 1997; Parinaud et al., 1999; Ali etal., 2000; Ali, 2004; Summers and Biggers, 2003; Matson and Tardiff, 2012; Sunde and Balaban 2013; Peirce et al., 2015, 2017 and others).

The risks associated with the use of biological components such as HSA or HSA-derived products as embryo culture media supplements is clear however it appears to be not viewed seriously as a threat to health by a vast majority of workers in the human assisted reproduction treatment industry. This is likely due to, firstly, a lack of a commercial source of a truly reliable and effective synthetic EM, which relies on donor protein components to fertilize human oocytes and to support the growth of resultant embryos in vitro. Efficacious chemically defined protein-free synthetic embryo culture medium has been developed (Ali, 1997, 2004; Ali et al., 2000) but as previously stated it remains to be manufactured on a commercial scale and marketed, therefore not available to the IVF fraternity. Secondly, on the surface at least, no tangible or dramatically disastrous harm had befallen healthcare workers in the area of assisted reproduction treatment or the embryos generated from culture systems that incorporated donor protein supplements albeit two previous reports (van Os et al., 1991; Kemmann, 1997) on transmission of pathogenic agents through donor protein supplement. Nevertheless the potential for the transmission of protein-bound pathogenic agents in culture media remains a real threat. It is important that this matter is viewed seriously because of the enormous potential for harm from tainted albumin due to the large number of people undergoing in vitro fertilization (IVF) treatment.

There are over 2 million IVF treatment cycles performed per year worldwide of which at least around 25% were successful, resulting in about 500,000 live-births (de Mouzon et al., 2020). It is readily obvious what harm can befall the patients and possibly the general public if an infectious pathogenic agent inadvertently was transmitted in the EM through tainted albumin supplement.

Most contemporary EMs with the possible exception of one EM described by Ali (Ali, 1997; Ali et al., 2000) contains undefined components from donor HSA or HSA-derived products which could contain potentially or in some instances evidently harmful components such as hazardous pathogenic agents (van Os et al. 1991; Kemmann, 1998) because biological components cannot be sterilized with absolute certainty (Truyen et al., 1995), donor micro strands of RNA/DNA well recognized to be present in HSA may pose risk of transcriptomic modification or imprinting disorders (Hirasawa and, Feil, 2010) and the undeclared inflammatory proteins could be potentially harmful to the embryo and babies born from affected embryos (Dyrlund et al., 2014). As previously stated undefined components naturally will confer batch inconsistency to the EM during production because each batch of biologically derived products will contain different spectra of contaminating components in minute concentrations. Obviously it is preferable to manufacture media that are devoid of undefined components that confer or demonstrate batch consistency as well as safety. To achieve this objective of batch consistency and safety, all components of the EM must be defined and or synthetic.

In addition to the potential of transmission of pathogenic agents through donor protein supplements, a number of other contaminant undeclared proteins, about 110 contaminant proteins, are also transmitted (Dyrlund et al., 2014) in the HSA and possibly also in HSA-derived products. Of these 110 contaminant proteins, 18 were associated with the innate immune response and 17 with inflammatory responses. These undeclared contaminant proteins could potentially influence embryonic development, gestation age, birthweight and perhaps have subsequent effects on health of the offspring (Dyrlund et al., 2014).

Cultural permissibility

This study provides evidence that the IUCS Another contentious issue is the report that suggests a model in which maternal endometrial microRNAs (miRNAs) act as transcriptomic modifiers of the pre-implantation embryo (Vilella et al. 2015) such that the modifications becomes evident and is expressed in the offspring carried to term. This came to the fore in women undergoing IVF treatment using donor eggs. In this scenario, infertile women who carry a child fertilized using a donor egg will still impart something of their own genetic information through transcriptomic modification brought about by their own endometrial miRNAs. The endometrial miRNAs in the vicinity of the embryo in utero gains access to the embryo to effect the transcriptomic modification This observation lends credence to the assumption of the authors that the same could occur with contaminant donor miRNAs transmitted into the EM by HSA or HSA-derived products. This would mean that the donor miRNAs could also likewise act as transcriptomic modifiers of the cultured pre-implantation embryos of IVF patients. If this occurs, the use of HSA in EM will not be acceptable to many cultures. Some cultures do not permit third party involvement in reproduction where the preservation of the purity of the lineage of the progeny is paramount. There is a need to comply with cultural norms and values.

Furthermore some cultures do not permit the use of blood or blood products. In such cultures the patients may object if they knew their embryos are grown in media containing donor serum proteins. Importantly, the patients are completely oblivious of the components used to manufacture the EM. They have no clue whatsoever of the biological and or the toxicological properties, and thus, of the issue of cultural permissibility of these components. Patients place complete trust in the healthcare system and workers, and expect to be informed of the hazards of the treatment procedure including matters that may contravene cultural permissibility.

Of considerable interest and significance is that the matter of cultural permissibility or inappropriateness of the components used in making the EMs. This has probably never been

conveyed to the patients. For instance caste Hindu vegetarians, some sects of Christianity, and other religious communities such as Muslims may not be satisfied, be offended or even express revulsion, if they knew their embryos are being nurtured and grown or handled in EM containing donor human serum proteins such as the human serum albumin (HSA) or HSA-derived products. This discussion raises the question of permissibility of use of HSA in the EM.

The question is do patients have the right to know their embryos are generated in EM that contains culturally non-permissible components? While the risks involved in the IVF treatment protocol are made known to the patients however the hazards, risks, and culturally inappropriate practices associated with the use of EMs containing donor serum proteins is hardly ever conveyed to them. To be fair the healthcare workers are not blameworthy because a vast majority of the healthcare workers themselves are completely unaware of the ingredients utilized to manufacture the EM. They in turn place complete trust in the regulatory authorities responsible for approving healthcare products or devices. Consequently, there is a sense, or more appropriately, a false sense of perceived safety and a culture of permissibility and acceptance among healthcare workers and, therefore it probably never occurred to them the need to discuss the matter of the cultural permissibility of EM, or its constituent components with their patients.

Discussion

Based on the literature, it appears with the passage of time, workers from later generations of embryologists are less vigilant, less vocal, or less watchful towards the hazards or inappropriateness associated with the use of donor proteins in EM. The good pregnancy rates achieved with protein-added contemporary media has all but stamped out debates or discussions on the hazards associated with the use of undefined EM. A vast majority of the fraternity appear to have accepted some the EMs of questionable safety, which have masked the issues of hazard of transmission of hazardous agents, cultural permissibility and batch inconsistency, which has ultimately led to a relegation of these issues to a status of practical insignificance which may prove detrimental in the long term.

Another question, although not within the scope of this discussion but worth a mention is whether the EMs are products of painstaking systematic research or were some of them, especially cryopreservation media, whimsically formulated? Unless the research protocol and experimental design used for the formulation of the various EMs are communicated, the fraternity cannot concede the media products in the marketplace are not whimsical. Whimsical formulations could negatively impact the treatment outcome and the long term health and well-being of individuals born of the embryos generated from such media. In recent years concern has been raised that end-users (that is the healthcare workers) of the EM must know its composition (Sunde et al., 2016) because the safety of IVF treatment with regard to the health of the offspring is of paramount importance.

Conclusion

Numerous potentially hazardous events could occur with the use of non-uniform albumin preparations in the EM. Furthermore culturally contentious issues of non-compliance with norms exists with the use of albumin in EM. In view of the hazards and disadvantages associated with the use of non-uniform albumin in EM it is suggested that efforts are made to utilize synthetic EM in assisted reproduction procedures.

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