Embryo culture media for human IVF: which possibilities exist?

İnsan İVF'i için embriyo kültür ortamı: hangi olasılıklar mevcut?

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Abstract

The last three decades have seen considerable progress in the development of culture media for ART and infertility treatment. Basic research on the metabolism of mammalian preimplantation embryos demonstrated the specific needs in the evolving stage of the embryo growing in vitro. Two different philosophies led to two different culture strategies for human preimplantation embryos: the 'back-to-nature' or sequential culture principle, and 'let-the-embryo-choose' or one-step culture principle. Both systems are commercially available and the discussion between the different groups of scientists is ongoing. As a matter of fact, all ART culture media currently used are not optimal for the growing human preimplantation embryo. However, further research is needed to reduce stress to the human preimplantation embryo and determine how many embryos from a treatment cycle are capable of producing a live birth.

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Key words: Culture media, back-to-nature, 'let-the-embryo-choose', media components

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Özet

Son 3 yılda ART ve infertilite tedavisi için kültür ortamı geliştirilmesinde önemli ilerlemeler görülmektedir. İmplantasyon öncesi memeli embriyosu metabolizması üzerine temel araştırma, in vitro gelişen embriyonun gelişim aşamalarındaki özgün gereksinimlerini gösterdi. İki farklı düşünce şekli implantasyon öncesi insan embriyoları için iki farklı kültür stratejisine yol açtı: 'doğaya dönüş' veya ardışık kültür ilkesi ve 'bırak embriyo seçsin' veya tek-adım kültür ilkesi. Her iki sistem de ticari olarak mevcuttur ve farklı gruplardaki bilim adamları arasındaki tartışma devam etmektedir. Gerçekte, şu an kullanımda olan ART kültür ortamlarının hiç biri implantasyon öncesi insan embriyoları için optimum değildir. Bununla beraber, implantasyon öncesi insan embriyosuna stresi azaltmak ve bir tedavi döngüsünden elde edilen embriyoların kaç tanesinin canlı bir doğum oluşturma becerisine sahip olduğunu belirlemek için daha fazla araştırmaya gerek vardır. (J Turkish-German Gynecol Assoc 2011; 12: 110-7)

Anahtar kelimeler: Kültür ortamı, 'doğaya dönüş', 'bırak embriyo seçsin', besiyeri bileşenleri

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Introduction

The goal of embryo culture in an assisted reproductive (ART) programme is to improve the quality of embryos developing in the laboratory and the chances of successful delivery of a healthy baby. Culture conditions for human embryos have evolved over the past thirty years (1-4).

Cleaving embryos normally develop in the fallopian tube, whereas the natural environment for morulae and blastocysts is the uterine cavity. Previously, it was conventional to use media permitting culture of human in-vitro fertilized embryos for 2 to 3 days to reach the four-to-eight cell stage, with additional embryo transfer to the patient (5). Premature replacement of the human embryo to the uterus may in part account for the low implantation rates associated with human IVF, with only approximately 10% of embryos transferred leading to a live birth. Further basic research on the metabolism of in-vitro fertilized embryos revealed that there are specific needs, depending on the developmental stage of the preimplantation embryo. In addition, improvements in culture media resulted from an increased understanding of the environment of the oviduct and uterus (6-8). Since 1997, the extended culture in sequential serum-free culture media has attracted more attention. The ability to culture zygotes to the blastocyst stage should help to synchronize the embryo with the female reproductive tract, and to help to identify those embryos with little development potential (3).

Evolution of embryo culture media

The idea introduced by Bernard in the late 1800s that the immediate environment surrounding living tissues is an active one led, in turn, to the notion that organs and tissues could be studied outside their setting in a suitable fluid formulated to facilitate these studies (9). Less than 10 years later, Ringer devised a solution of salts still used today in surgical treatments. It is an interesting aspect that the culture media evolved and used in the clinical setting were construed to support the development of somatic cell culture applications. The first success of fertilization of the human oocyte in vitro by Robert Edwards was accomplished in a simple, chemically defined media. These commercially available media were a modified Earle's balanced salt solution, and a modified Ham's

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F10 or T6. They were supplemented with maternal serum thus converting them into biological media (10, 11). Menezo et al. (12) broke with the tradition of using balanced salt solution and produced a medium containing amino acids without the need of a serum supplement. Another medium specifically designed for human IVF was human tubal fluid (HTF) (13). Human tubal fluid, supplemented with either whole serum or with serum albumin, gained great popularity for the use of day 2 or day 3 human embryo cultures, and has remained in use ever since.

A culture medium is a foreign environment for the human embryo. Hence, the design of media is complicated, because the components must be selected, and their concentrations determined in order to minimize stress for the cultured embryo (2, 14, 15). It also became clear that early embryos show an evolving need for energy substrates, moving from a pyruvate-lactate preference - while the embryos are under maternal genetic control - to glucose-based metabolism after activation of the embryonic genome (7, 16). Two investigators responded to these findings by modifying the HTF media. Quinn (17) removed glucose and inorganic phosphate, QB 11, for obtaining the first glucose-free medium. Pool (18) also generated a HTF variant, called Preimplantation Stage 1 or P-1 medium, a glucose- and phosphate-free medium, but additionally containing the amino acid taurine. The improved understanding of both the physiological changes in oviduct and uterus (7) and the different metabolic needs of the cleavage-stage and blastocyst-stage embryo led to the development of stage-specific or "sequential" complex media G1/G2 (3). Barnes et al. (19) used this combination to produce pregnancy and live birth after the transfer of a single viable human blastocyst, and the media were first slightly modified and marked as the GIII/G5 series of media. Other popular sequential systems, such as Quinn's series in the United States and the MediCult/Origio media from Europe and Cook from Australia, are also in widespread use. Lawitts and Biggers (20, 21) broke new ground to design a chemically defined media. They applied the principle of simplex optimization to determine the optimal concentration of each media component. This resulted in the formulation of Simplex Optimizition Medium (SOM). SOM has been modified in several ways to mKSOM^{AA}. Henceforth, it is possible to provide a one-step protocol (so-called global medium) to culture human zygotes to the blastocyst stage (2, 5). Now, there exist three commercially available one-step human embryo culture media (global®, LifeGlobal, U.S.; Gynemed GM501®, Lensahn, Germany; SSM™, Irvine Scientific, U.S.). At least nine companies now advertise media for the culture of human preimplantation embryos to the blastocyst stage (Table 1).

The recovery of immature oocytes followed by in-vitro maturation (IVM) of these oocytes led to the development of specific conventional available IVM culture media.

Two philosophies for human embryo culture media

The design of media for the culture of preimplantation embryos has been influenced by two fundamentally different philosophies (2, 22). However, growth of ART embryos is inferior to that of in vivo embryos, indicating that ART procedures invoke cellular and metabolic stress situations and the ART embryo is forced to spend energy to adapt to this foreign environment. In particular, the culture media is an important factor in successful in vitro interactions between gametes and subsequent embryo development (3). Manufacturers of human embryo culture media follow either the "back-to-nature" (sequential media) or the "let-the-embryo-choose" (global media) philosophy (23). The key components of both modern media are shown in Table 2.

The sequential culture-"back-to-nature" principle

The "back-to-nature" attempts to mimic the changing needs of the developing zygote and embryo in a media should approximate the concentration to which the embryo is naturally exposed (3, 23). The embryo is capable of actively controlling ionic gradients etc, and is able to regulate its internal environment. Therefore, with regard to embryo physiology, it is appropriate to consider the preimplantation period in at least two phases: pre- and post-compaction (3). Such a breakdown of the preimplantation period is of importance when one considers changes to medium formulations. Other considerations include the time at which the embryonic genome is activated (3).

The monoculture "let-the-embryo-choose" principle

The design of a culture medium involves the simultaneous use of all the concentrations in a mixture because the effects of each component in the medium may depend on the concentrations of the other components (23). As long as concentrations are within 'tolerable ranges', the embryo itself will adapt and utilize whatever it requires (2, 23, 24). This philosophy led to a family of media in which all of the substances necessary to early embryological development are provided, and there is no need for a media change. One-step formulation is applied throughout the entire in-vitro development from fertilization to the blastocyst stage of the embryo.

Four protocols can be used for the culture from fertilization to the blastocyst stage in an ART laboratory: [a] sequential media protocol, with an interrupted culture where two media of different compositions are used sequentially, change of medium occurs on day 3 of embryo culture, [b] sequential media protocol with fresh medium change every day, [c] monoculture, uninterrupted culture using one medium throughout the 5 days of embryo culture, [d] interrupted culture where a monoculture medium is used throughout but is renewed on day 3 of embryo culture.

Key components of ART culture media

Studies using the development of mammalian preimplantation embryos in-vitro have played a major role in the understanding of pre-embryo physiology (for reviews, see 3, 24-30). As a result, this is also the limitation in the development of culture media for the human embryo. The most widely used models for the human embryo have been the mouse and the cow.

Carbohydrates

In brief, the early embryo shows a rather simplistic physiology and maintains only low levels of oxidative metabolism, whereas it exhibits a somatic-cell like physiology after compaction

Table 1. Available commercial systems for human IVF culture

One media system			
Company	**Medium**	**Culture period**	**Website**
LifeGlobal	global®	day-1 to day-5/6	www.lifeglobal.com
Gynemed	GM501	day-0 to day-5/6	www.gynemed.de
IrvineScientific	SSM™	day-0 to day-5/6	www.irvinesci.com
Sequential media system			
Company	**Medium**	**Culture period**	**Website**
Cook Medical	Cleavage K-SICM	day-1 to day-3	www.cookmedical.com
	Blastocyst K-SIBM	day-3 to day-5/6	
CooperSurgical	Quinns Advantage®Cleavage	day-1 to day-3	www.coopersurgical.com
	Quinns Advantage®Blastocyst	day-3 to day-5/6	
FertiPro	FERTICULT™IVF Medium	day-1 to day-2	www.fertipro.com
	FERTICULT™ G3 Medium	day-3 to day-4	
InVitroCare	IVC-TWO™	day-0 to day-3	www.invitrocare.com
	IVC-THREE™	day-3 to day-5	
Irvine Scientific	ECM®	day-0 to day-3	www.irvinesci.com
	MultiBlast®	day-3 to day-5	
Origio	EmbryoAssist™	day-0 to day-3	www.origio.com
	BlastAssist™	day-3 to day-5	
	ISM1	day-0 to day-3	
	ISM2	day-3 to day-5	
Vitrolife	G-1™PLUS	day-1 to day-3	www.vitrolife.com
	G-2™PLUS	day-3 to day-5	
	IVF™	day-0 to day-3	
	CCM™	day-3 to day-5	

utilizing a wider spectrum of nutrients, biosynthetic rates are increasing, along with an increased respiratory capacity and an ability to utilize glucose (8, 28). This involves a shift in the energy requirements at the time at which the embryonic genome is activated or at the post-compaction stage. Zygotes and subsequent cleavage stages prefer pyruvate as the primary source of energy, while the eight-cell-stage embryo uses glucose (31-33). Glucose is a key anabolic precursor and is required for the synthesis of triacylglycerols and phospholipids, and as a precursor for complex sugars and glycoproteins. Glucose also metabolized by the pentose phosphate pathway (PPP) generates ribose moieties required for nucleic acid synthesis (34).

Amino acids

It has been proposed that "amino acids-(AA)", a term which includes all 20 common and naturally occurring amino acids, are important regulators of mammalian preimplantation development (for reviews, see 8, 29, 35). Prior to embryonic genome expression, the embryo utilizes carboxylic acids and AA as energy sources (29). In addition, certain AA are known to function as biosynthetic precursor molecules (36), osmolytes (37), buffers of internal pH (38), antioxidants (39) and chelators, especially for heavy metals (40). It is important to note that there are also specific changes in the nitrogen requirements of the embryo (27, 28). The seven non-essential AA and glutamine stimulate the growth of the early cleavage embryo (41). In contrast, an inhibitory effect was seen on blastocyst development and viability if the thirteen essential AAs are presented at an early stage (42). At the post-compaction stage, both groups of AAs act stimulatory to the inner cell mass of blastocysts, while the non-essential AAs and glutamine lead to stimulation of the throphectoderm and hatching from the zona pellucida (43, 44). Leese at al. (45-47) have described AA turnover studies on the mammalian embryo and argued for "quiet" embryo metabolism during preimplantation embryo culture and development to produce the most viable embryos.

However, AAs in culture media also spontaneously undergo breakdown to release ammonium into the culture medium with concentration being time dependent. Ammonium is toxic to the embryo and reduces viability (48). Especially L-glutamine (Gln) is highly unstable in solution, where it breaks down fairly rapidly into equimolecular amounts of ammonium and pyrrolidine-5-carboxylic acid (for review see, 49). Therefore, Lane and Gardner (42) introduced a two-step (sequential) cul-

Table 2. Key components of modern media

Components	One media system Gynemed GM501®	Sequential media G-1™PLUS	Sequential media G-2™PLUS
Salts	Sodium chloride	Sodium chloride	Sodium chloride
	Potassium chloride	Potassium chloride	Potassium chloride
	Calcium chloride	Calcium chloride	Calcium chloride
	Monopotassium phosphate	Sodium citrate	Sodium citrate
	Magnesium sulphate	Magnesium sulphate	Magnesium sulphate
		Sodium dihydrogen phosphate	Sodium dihydrogen phosphate
Buffer	Sodium bicarbonat	Sodium bicarbonate	Sodium bicarbonate
Energy Substrates	Glucose	Glucose	Glucose
	Sodium lactate	Sodium lactate	Sodium lactate
	Sodium pyruvate	Sodium pyruvate	Sodium pyruvate
Non-Essential AA's	NEAA's	8 NEAA's	9 NEAA's
Glutamine Dipeptide	Alanyl-Glutamine		
Essential AA's	EAA's	2 EAA's	11 EAA's
Chelator	EDTA	EDTA	none
Macromolecules	none	Hyaluronan, HSA	Hyaluronan, HSA
Fatty acid	none	Lipoic acid	none
Vitamins	none	none	4 Vitamins
Indicator	Phenol Red optional	none	none
Antibiotic	Gentamicin	Gentamicin	Genamicin
Water	yes	yes	yes

ture media protocol to remove the accumulated ammonium. Another possibility is replacing Gln with a stable dipeptide of Gln (24). It must be noted that culture media should include sufficient levels of sulphur containing amino acids to minimize apoptosis leading to monozygote twinning (50).

EDTA (Ethylenediaminetetraacetic acid)

Its usefulness is based on its role as a ligand and chelating agent, i.e. its ability to "sequester" metal ions. After being bound by EDTA, metal ions remain in solution but exhibit diminished reactivity. The addition of EDTA to culture media alleviates the 2-cell block in mice embryos (51) and inhibits premature utilization of glycolysis by cleavage stage embryos, thereby preventing any Crabtree-like effect that is associated with arrest in culture (48). However, EDTA at a concentration of 0.1mmol/L reduces blastocyst development and cell number (52). Other investigators indicated that an EDTA concentration of 0.005-0.01 mmol/L did not have a deleterious effect on murine preimplantation or postimplantation development (53).

Regulation of cell volume-osmolytes

Maintenance of a constant volume in the face of extracellular and intracellular osmotic perturbations is a critical problem faced by all cells. Most cells respond to swelling or shrinkage by activating specific metabolic or membrane-transport processes that return cell volume to its normal resting state. These processes are essential for the normal function and survival of cells (54). The osmotic pressure of oviduct fluid is >360 mOsmol (55). However, the osmolarity of most commercially available ART culture media is lower-at about 250-300 mOsmol. When the NaCl concentration is forced up to 290 mOsmol, the development of the embryo is severely impaired (56). Addition of extracellular AA, such as glycine, betaine, proline, alanine and hypotaurin which act as organic osmolytes, protects the preimplantation embryo against hypertonicity and increases embryo development (37, 56, 57).

Impact of pH and buffers

The pH only refers to hydrogen ion concentration and is only meaningful when applied to aqueous (water-based) solutions. When water dissociates it yields a hydrogen ion and a hydroxide ion, H_2O <--> $H^+ + OH^-$ (for review see, 58, 59). It must be noted that pH is dynamic. The balance of pH depends on the association or dissociation of compounds. The most important ions are sodium, potassium, magnesium, chloride and lactate and also the AA glycine which acts as an intracellular zwitterionic buffer (60). An acceptable pH range for embryo culture media may be set between pH 7.4 and 7.2. Culture media pH is regulated by the balance of CO_2 concentration, supplied by the media and by the concentration of bicarbonate in the media. However, the intracellular

pH in human cleavage embryonic cells is pH=7.2 (61) and pH is an important cellular function which is necessary to maintain intracellular homeostasis. Moreover, after the compaction stage the preimplantation embryos appear to have more control over their intracellular pH, because of the formation of tight junctions between cells (38, 62). Hence, there is a trend to culture cleavage stage embryos in a slightly lower pH and morulae and blastocysts in a slightly higher pH (low-high paradigm). Table 3 provides information about the recommended pH of commercial available media.

In the past, handling media were used with phosphate-buffered saline solutions (PBS) or different "Good's" buffers (63). Nowadays, especially two "Good's" puffers are used in commercially IVF handling media. The most commonly used buffer is 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES at 21 mmol/L), whereas some companies include 3-(N-morpholino)-propanesulphonic acid (MOPS). Both buffers have a pK_a value of 7.2, it is the closest of the zwitterionic buffers to the pH_i of embryos of 7.12.

Although both buffers have been widely used in IVF handling, studies indicated there may be species specific sensitivities to HEPES (64, 65). Results demonstrated that, in the presence of optimal culture conditions, such as pH, gas concentration, osmolarity etc., HEPES is able to support mammalian embryo development and can also act as a chelator of heavy metals such as copper (66). If using MOPS for IVF handling at 37°C the pK_a for this buffer is actually 7.02 (59), which is low, because most IVF laboratories target their media pH at 7.3. Yet, MOPS can interact with DNA in cellular preparations (67). Currently, it has not yet been defined whether both buffers used have an impact on embryo osmotic regulation (59).

Table 3. Recommended pH-ranges of IVF culture media (adapted from Swain, 2010)

Company	Medium	pH-range
LifeGlobal	global®	7.2-7.4
Gynemed	GM501	7.2-7.4
Irvine Scientific	SSM™	7.28-7.32
	ECM®	7.2-7.25
	MultiBlast®	7.3-7.4
Cook Medical	K-SICM	7.3-7.5
	K-SICB	7.3-7.5
Cooper Surgical	Quinns Advantage®Cleavage	7.1-7.3
	Quinns Advantage®Blastocyst	7.2-7.4
FertiPro	FERTICULT™IVF Medium	7.2-7.6
	FERTICULT™G3 Medium	7.3-7.6
InVitroCare	IVC-TWO™	7.25-7.45
	IVC-THREE™	7.25-7.45
Origio	EmbryoAssist™	7.3-7.5
	BlastAssist™	7.3-7.5
	ISM1	7.2-7.4
	ISM2	7.2-7.4
Vitrolife	G-1™PLUS	7.27±0.07
	G-2™PLUS	7.27±0.07
	IVF™	7.35±0.10
	CCM™	7.35±0.10

Macromolecules

Common sources for macromolecules are proteins for culture media such as human serum albumin or synthetic serum. Both are added at concentrations of 5 to 20%. Today, most commercial media include synthetic serum in which the composition is well known. Protein in the form of albumin is thought to maintain the stability of cell membranes and chelate trace amounts of toxic components presented in culture water, media components and culture dishes. Other functions include capillary membrane permeability and osmoregulation. The presence of macromolecules in embryo culture media serves to facilitate manipulation of gametes and embryos (8). However, the uses of any blood products involve the risk of potential contamination and infection of preimplantation embryos.

Some investigators have used synthetic polymers such as polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP) in ART (29) but neither can be considered a physiological alternative to protein (68).

Another physiological alternative to albumin is the glycosaminoglycan hyaluronate (also called hyaluronic acid or hyaluronan). The human embryo expresses the receptor for it throughout preimplantation development (69). While hyaluronate could not only replace serum albumin in culture, it increased the implantation rate of resultant mouse embryo blastocysts (70). Therefore, hyaluronate can replace albumin as a sole macromolecule in an embryo transfer medium and in some infertile patients it can improve ongoing pregnancy rates (71).

Vitamins

The addition of vitamins as antioxidants to the culture media containing glucose and phosphate helped to prevent a loss in respiration and metabolic control (72). The following possible vitamins are components of different ART culture media: ascorbic acid, cyanocobalamin, folic acid and tocopherol. Their optimum concentrations were determined using mouse zygote assays. Moderate dosages of vitamins C and E were seen to reduce oxidative damage in mouse embryo culture and improve their blastocyst development rate (73).

Growth factors

Mammalian embryos are naturally exposed to a complex mixture of growth factors that play a key role in growth and differentiation from the time of morula to blastocyst transition. However, defining their role and potential for improving in-vitro preimlantation development is complicated by factors such as gene expression of both the factors and their receptors. The blastocyst expresses ligands and receptors for several growth factors, many of which can cross-react thus making it difficult to interpret the effect of single factors added to a culture media (74, 75).

Antibiotics

Embryo culture media are routinely supplemented with antibiotics to prevent bacterial contamination (76). Nowadays, commonly used antibiotics are penicillin (β-lactam; 100U/ml), streptomycin (aminoglycoside; 100 μg/ml) and gentamycin (aminoglycoside; 50 μg/ml). The anti-bacterial effect of penicillin is attributed to its disturbance of cell wall integrity through the inhibition of the synthesis of peptidoglycan. Penicillin has no direct toxic effects on the preimplantation embryo. Streptomycin and gentamycin disturb bacterial protein synthesis. However, the aminoglycosides show more toxic effects (76).

Literature review for comparison of media types

A number of recent studies have been conducted to compare the effectiveness of commercially available ART culture media types. A search was conducted on published literature. Interestingly, most studies prefer 3-day human IVF embryo culture and embryo transfer for comparison of different media types (77-81). Differences in embryo quality were observed in studies that used modern formulated media versus standard media, but no differences in pregnancy rates were reported (77, 78). Moreover, no differences between a single or one-step defined medium versus a cleavage-stage media with regard to fertilization, pregnancy implantation rates, and ongoing pregnancy were found by following studies (78, 81). Ebert et al. (79) reported similar results. Only the rate of pregnancy losses was significantly lower in patients with the one-step medium GM501 as compared to the Universal IVF medium.

Three studies assess pregnancy outcomes and embryo morphology after transfer of day-3, day-5 or -6 embryos (82-84). Van Langendonckt et al. (82) matched two sequential media, G1.2/G2.2 and Sydney IVF cleavage/blastocyst media. Both media yielded similar outcomes in the blastocyst transfer programme, but a lower day-3 embryo quality in the G1.2 media. The other two studies compared a single-step medium versus a sequential medium. Reed et al. (83) reported no significant difference for results on day-3 transfer. However, for day-5 transfer, a greater number of blastocysts were available from the single medium. Paternot et al. (84) described similar positive results using GM501.

Two other studies compared a single-step versus a sequential media system for the development of the human embryos to the blastocyst stage (85, 86). Biggers and Racowsky (85) found that significantly more IVF-embryos cultured in the single-step medium showed cytoplasmic pitting. IVF-blastocyst formation rates were not significantly different between the two media systems. Sepulveda et al. (86) referred to donor cycles, and had better development rates on days 3, 4 and 5 as well as significantly higher implantation rates for embryos cultured in the single medium.

Furthermore, three other studies used the mouse model for comparison of commercially available media (53, 87, 88). Biggers and colleagues (53) observed no significant differences in the proportion of the blastocysts, rates of hatching, numbers of cells in the inner cell mass and trophectoderm between KSOM^{AA} and G1.2/G2.2. However, Perin et al. (87) reported a higher blastocyst formation, higher cell numbers in the inner cell mass and higher hatching rates after culture in the single-step medium KSOM^{AA}. Hentemann and Bertheussen (88) compared two sequential media, BlastAssist M1 and M2 versus G1/G2, in a mouse model and achieved similar results.

Concluding remarks

In this review, two different types, 'back-to-nature' and 'let-the-embryo-choose', of culture media were presented with the recent literature. Both media philosophies are part of worldwide practice in the ART laboratories. Based on recent literature, it can be concluded that global one step media are at least as useful as sequential media.

Human embryos can develop in vitro in rather different types of media from basic systems to sequential complex culture media. ART culture media contain only a subset of parts which are found under in vivo conditions. Hence, embryos cultured in-vitro was exposed to constant stress. Suboptimal culture conditions force the embryo to undergo adaptations, and thus lead to lower pregnancy and higher abortion rates.

It is evident that all necessary steps in ART as part of the treatment of infertility can influence the epigenetic programming during early development (89). Therefore, it is essential that a high level of quality control exists in the laboratory, and it is suggested that further investigations are necessary to optimize environmental conditions in which the preimplantation embryo can evolve.

Conflict of interest

No conflict of interest was declared by the authors.

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