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REVIEW

In-vitro culture of human embryos with mechanical micro-vibration increases implantation rates

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Abstract The in-vitro culture of human embryos in a medium subjected to regular short intervals of mechanical agitation leads to increased development rates. This type of treatment tries to mimic conditions in nature whereby oviductal fluid is mechanically agitated by the epithelial cilia. This phenomenon can be explained by the fact that an embryo developing *in vivo* is naturally exposed to constant vibrations of around 6 Hz with the periodically repeating increase to 20 Hz. This review covers the history of this question and in this light offers an explanation through biological concept for one of the most recent developments in this area: in-vitro culture of human embryos with mechanical micro-vibration. The effect of mechanical micro-vibration on embryos during their in-vitro culture was examined. Pregnancy rates after the transfer of embryos in the group with in-vitro culture under mechanical vibration were increased.

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In-vitro culture system for oocytes and embryos: permanent process of improvement

Early attempts to culture cleavage-stage rodent embryos in a completely in-vitro system were met with limited success (Defrise, 1933; Mark and Long, 1912; Tarkowski, 1962; Washburn, 1951; Warwick and Berry, 1949). Subsequent attempts worldwide to improve the in-vitro culture systems used have mostly centred on modifying the components of soluble media such as salts, energy or nitrogen sources, and growth factors/hormone supplements were quickly to become the main focus of such investigations (Biggers and Summers, 2008; Loutradis et al., 2000; Summers et al., 2005).

Regardless of the type of medium and culture volume, all culture systems can be described as a standard static

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culture system comprising a small (a few microlitres) or not so small (up to 1000 μl) volume of culture medium (Brison et al., 2004; Thompson, 2007; Gardner and Lane, 2000). These culture conditions are, however, far from natural. In a recent review (Fauci and Dillon, 2006), the mechanism of reproduction was described as highly complex involving the concerted actions of motile spermatozoa and muscle contractions of the uterus and oviduct, as well as ciliary beating at the moment of fertilization and during embryo transport via the oviduct to the uterus. During this time period (around 5–7 days), the cilia generate forces that drive fluid motion, but their configurations are, in turn, given by the fluid dynamics (Fauci and Dillon, 2006). All this creates a dynamic culture system in which the embryo develops (Foo and Lim, 2008; Muglia and Motta, 2001). The dynamics of culture systems can be intensified by chemical factors (Hardy and Spanos, 2002; Muglia and Motta, 2001) and can lead to an increase in the positive effect of hormones and nutrients on the eggs or embryos (Anand and Guha, 1978; Blake et al., 1983).

It is well known that both the micro- and macroenvironment play an important role in determining the developmental competence of an embryo cultured *in vitro* or grown *in vivo*. Research efforts to improve this microenvironment have involved two main strategies. The first of these is the development of a static culture system in which single cells or embryos are embedded in a gel (for example, natrium alginate) to isolate them from the possible detrimental effects of the surrounding macroenvironment (Torre et al., 2007), or an artificial microenvironment is created by culturing embryos in special micro-wells at the bottom of culture dishes (Hoelker et al., 2009; Vajta et al., 2000). The second strategy pursued has been the development of a dynamic culture system in which, as before, single cells are embedded in an agar gel to separate them from detrimental factors of the surrounding macroenvironment (Willadsen, 1979) and a perfusion system created in which nutrients circulate only around the cultured tissues/cells (Folkman et al., 1966; Rose, 1967).

The environmental conditions provided by circumfusion systems are highly favourable for maintaining differentiated chick embryo tissues over protracted periods (Rose, 1967). Recent studies have supported previous results indicating that the in-vitro culture of embryos using microfluidic devices (microchannels containing submicrolitre amounts of nutrient media) gives rise to lower percentages of degenerated embryos (Raty et al., 2004).

At the same time, this type of static microfluidic culture system has not been shown to improve pregnancy rates. Subsequent investigations addressing the use of a dynamic microfluidic culture system with continuous media perfusion for embryo culture have reported poor embryo development rates (Hickman et al., 2002) possibly due to shear stress (Walker et al., 2004) or the removal of autocrine growth-promoting factors (Paria and Dey, 1990). In a recent publication (Heo et al., 2010), a new microfunnel device that mimics body motions was found to overcome the negative effects of microfluidic culture systems (Paria and Dey, 1990; Walker et al., 2004). This new culture system results in significantly improved blastocyst cell numbers, developmental stage blastocysts and percentages of hatching or hatched blastocysts, as well as implantation and pregnancy rates (Heo et al., 2010). Despite such promising results, the procedure and microfunnel culture device is complex and would need to be simplified for routine use in IVF programmes.

In-vitro culture: artificial static versus natural dynamic

Since the birth of Louise Brown, the first baby conceived by IVF (Steptoe and Edwards, 1978), the knowledge of reproductive medicine has expanded rapidly. After this breakthrough, the main goal of reproductive biology has been the in-vitro production of high-quality preimplantation embryos. Routine assisted reproductive technologies today involve the use of static culture systems. However, in natural conditions (*in vivo*), the egg, spermatozoon and embryo are subjected to ever changing dynamic processes. In the popular system whereby embryos are cultured in small droplets, for example, toxic substances such as oxygen-derived radicals (Johnson and Nasresfahani, 1994) and ammonia (Gardner and Lane, 1993), with known detrimental effects on embryos (Lane and Gardner, 2003), are likely to build up. The main principle of such a culture system is refreshment of the medium. However, although periodic media changes may prevent toxins accumulating, this manipulation leads to an imbalance with the elimination of beneficial auto- and paracrine factors (Fukui et al., 1996). The development of the embryo in the natural conditions, as it passes through the oviduct, is accompanied by the constant removal of metabolites, gas exchange and exposure to numerous factors, absent *in vitro*, that mediate maternal–embryonic communication (Hill, 2001; Paria and Dey, 1990). This difference in conditions could be responsible, at least in part, for the impaired in-vitro development of preimplantation embryos (Harlow and Quinn, 1982).

When a static culture system is used, the only thing that will prevent the deleterious effects of metabolites on the embryo is replacing the culture medium with fresh medium. The preimplantation mammalian embryo is a relatively autonomous system. Such a self-controlling system can regulate its own cell division and differentiation without being in contact with the maternal reproductive tract (Schultz and Heyner, 1993). Thus, *in vitro* this self-controlling system produces diffusible embryotrophic paracrine/autocrine factors, such as platelet-activating factor, which are in part responsible for the regulation of early embryo development (Gopichandran and Leese, 2006). The data from this study shows that the distance between individual bovine embryos in culture affects preimplantation development, especially blastocyst formation, cell number and metabolism. During in-vitro culture, often when refreshing the culture medium, besides unwanted embryo-metabolites, embryotrophic paracrine/autocrine factors are also removed.

Natural in-vivo culture: it is constant movement

All fertilization steps in nature (receiving the ovulated oocytes, providing a suitable environment for fertilization) and embryo development with subsequent transport to the

uterus take place inside the Fallopian tube. This multifunctional organ is mechanically active and undergoes two types of movement: muscular and cellular. The movement of the ovum changes to a complex pattern of rapid forward and backward motion associated with segmental contractions of the oviduct wall. The purpose of these muscular (peristaltic) and segmental muscular contractions may be to stir the tube's contents and ensure the mixing of gametes and embryos with tubal secretions (Muglia and Motta, 2001). The tubal mucosa is arranged as longitudinal folds and consists of a single layer of cuboidal or columnar epithelium.

The major cell types of this epithelium are secretory and ciliated cells (Lyons et al., 2006), whose cilia have a vibrating, or beating, action towards the uterus (Chauveau et al., 1973). Some authors have shown that this ciliary motion is determined by the stage in the menstrual cycle. Thus, a significant increase in the frequency of ciliary movement has been detected in the human isthmus and ampoule after ovulation (Critoph and Dennis, 1977). Further, recent investigations based on analogue contrast enhancement have shown an increase in ciliary beat frequency to 5.8 ± 0.3 Hz in the fimbrial section of the tube during the secretory phase (Lyons et al., 2002) compared with the proliferative phase (4.9 ± 0.2 Hz).

Ciliary vibration in the oviduct

'Ciliary' refers to the cilia, which is Latin for vibrating hairs. Baseline cilia beating frequencies have been reported to vary widely between individuals in the range of 5–20 Hz (Paltieli et al., 1995; Westrom et al., 1977). At the higher frequencies, mechanical contact between oocyte/embryo during the secretory phase also increases significantly. The ciliary beat has the following characteristics: (i) its rate is remarkably uniform (Borell et al., 1957); and (ii) the beat of a particular cilium and its adjacent cilium appears to be well co-ordinated and a definite metachronal wave is established (Holwill, 1974). This metachronism is defined as co-ordinated oscillation including a definite phasing of micro-vibration between the cilia of a single cell and a definite phasing of this vibration between the cilia of adjacent cells. The fluid that surrounds the cilia and forms a blanket above the tips of the cilia is a suspension of mucus (Miller, 1968).

Hence, the oviduct may have a significant influence on embryo development due to the following factors: (i) shear stress by tubal fluid flow; (ii) compression by peristaltic tubal wall movement; (iii) buoyancy; and (iv) kinetic friction forces between the embryo and cilia (Xie et al., 2006). All these factors lead to a an average ovum velocity of 0.1 $\mu m\ s^{-1}$ and, during strong wall contractions, reducing lumen size to less than 160 μm, i.e. close to ovum size, this velocity may increase to 3.8–6.8 $\mu m\ s^{-1}$ (Greenwald, 1961). Fluid movements produced by wall peristalsis are similar to those induced by beating cilia and attain an average velocity of 8.6 $\mu m\ s^{-1}$ (Anand and Guha, 1978). However, as the embryo moves through the oviduct towards the uterus so-called 'punctuate' velocities are recorded. According to non-published data (H. Croxatto, personal communication, in Xie et al., 2006), velocities of the embryo due to these punctuated fluid speeds could range from 6.5 to 29.7 $\mu m\ s^{-1}$. Notwithstanding, such low or high velocities attained for short periods do not harm embryos *in vivo* (Xie et al., 2006, 2007).

In-vitro culture with micro-vibration mimics in-vivo culture

Recreating the in-vitro conditions of the oviduct is not an easy task. For example, fluid movement and mechanical agitation of embryos during culture can disrupt concentration gradients of substrates, secretory molecules, dissolved gases and waste products. Although concentrations of gases, substrates or metabolites are difficult to measure in culture systems, one can appreciate that such disruptions of gradients could occur with media flow and embryo agitation (Heo et al., 2010). On the other hand, movement may ensure that unstirred layers do not form around the embryo and may facilitate exchange of gases and/or metabolites. Another potential problem is shear stress produced as a function of the velocity of the embryo and flow, and it has been proposed that the velocity of embryos should mimic the velocities achieved in the oviduct (Matsuura et al., 2010). In effect, a microfluidic dynamic embryo culture system with flow has recently been reported to improve mouse embryo development (Heo et al., 2010).

The important role played by the physical/mechanical environment in embryo development has been highlighted by work conducted by Smith's group (Heo et al., 2010) in which using a dynamic microfluidic in-vitro culture system for 48 h, but not 96 h, of pulsate media flow was found to improve the blastocyst cell count over that recorded after static culture. These findings indicate that the duration of pulsating media flow is important, but that it is not developmental-stage specific. These results have far-reaching implications since they indicate that such a culture system is not harmful to the biology of the zygote, which is developing intensively during the first 48 h of culture (Ma et al., 2001; Schultz, 1993). Accordingly, pulsate media flow culture would not be expected to be harmful and not specifically affect subsequent preimplantation developmental events such as compaction at the 8-cell stage and blastocyst formation. Using this system, the following results have been obtained: (i) hatching or hatched blastocyst rates of 31% for microdrops, 23% for microfunnels and 71% for microfunnel-pulsatile systems ($P < 0.01$); and (ii) average numbers of cells per blastocyst of 67 ± 3 for microdrops, 60 ± 3 for microfunnels and 109 ± 5 for microfunnel-pulsatile systems compared with 144 ± 9 for in-vivo grown blastocysts. Collectively these results suggest that the benefits of microfluidic-generated pulsatile fluid flow for embryonic developmental competence may be due to a culture microenvironment that is closer to in-vivo conditions of development (Heo et al., 2010). The authors of this study indicated that the microfunnel pulsatile embryo culture system provided periodic medium refresh, moderate retention of biomolecules and agitation of fluid surrounding the embryo (Heo et al., 2010).

Another system of in-vitro embryo culture, known as the tilting embryo culture system (TECS), attempts to deliver to developing mouse embryos similar mechanical stimuli to

those experienced in the Fallopian tube (Matsuura et al., 2010). A conventional culture dish is placed on a tilting platform such that the embryos in culture slide across the bottom of the dish due to gravity. In this study, the speed of tilting was adjusted to mimic the velocity ($\sim$1 mm/min) and shear stresses ($\leq$1.2 dyn/cm^2) of the mouse embryos in the oviduct. Tilting embryo culture system (TECS) was found to significantly improve blastocyst development rates of the mouse embryos (TECS 59% versus control 46%; $P < 0.05$). The number of cells in the blastocysts cultured using TECS was 77 ± 4 versus 66 ± 4 for controls ($P < 0.05$). For thawed human embryos, development rates to the blastocyst stage recorded in the TECS and control groups were 53% and 45%, respectively. The mean cell number of developed blastocysts on day 5 of in-vitro culture using TECS was 43 ± 3 compared with 34 ± 3 for controls ($P < 0.05$). TECS was also found to significantly improve the development of low-quality human embryos and the development of mouse embryos cultured in suboptimal conditions. According to the authors, a possible reason for such improvements in both mouse and human embryo development could be mechanical stimuli produced by embryo motion (Matsuura et al., 2010) suggesting benefits of the clinical use of TECS.

The above findings support the results of prior studies in which ovarian tissue pieces were cultured *in vitro* using a dynamic culture system (Isachenko et al., 2006, 2007, 2008a,b,c, 2009a,b,c,d, 2010a,b). Fragments of ovarian tissue were cultured for 2—6 weeks in a large volume of medium in a culture flask that was subjected to 75 oscillations/min on a rotatory shaker. Compared with the static culture system, the follicles inside these tissue fragments returned high viability and development rates. The goal was to develop a system that could offer the combined benefits of the microfunnel pulsative and tilting embryo culture systems.

In-vitro culture system with pulsative mechanical micro-vibration

Initially, the information reporting the benefits of pulsative mechanical micro-vibration for the cytoplasmic maturation of in-vitro matured pig oocytes was published by Miyoshi's group (Mizobe et al., 2010). These authors subjected cumulus—oocyte—complexes cultured in micro-drops to pulsatile mechanical vibration (PMV) at a frequency of 20 Hz with acceleration (660 mV/g at 3.3 V: X = ±1.0g, Y = ±0.7g, Z = ±0.15g; instruction of manufacturer). During in-vitro maturation, PMV did not affect the proportion of oocytes reaching the metaphase-II stage. However, blastocyst formation rates after the activation of oocytes exposed to PMV were significantly higher ($P < 0.05$) than those obtained for oocytes matured without mechanical vibration (27% versus 12% and 26 versus 15%, respectively, for the 5 s and 10 s pulses).

The question then asked was: why did these authors choose a micro-vibration frequency of 20 Hz? The reply to this question is based on: (i) the significant increase in ciliary beating frequency of the human isthmus and ampoule produced after ovulation, that is, in the secretory phase of the oestrus cycle (Critoph and Dennis, 1977; Lyons et al., 2002); and (ii) the baseline frequency of ciliary beating varies among individuals from 5 to 20 Hz (Paltieli et al., 1995; Weström et al., 1977).

In-vitro culture of human embryos with mechanical micro-vibration: own experience

The current work was performed at a private medical centre (Endokrinologikum Ulm, Praxisklinik Frauenstraße, Ulm, Germany, www.kinderwunsch-ulm.de). Couples were offered the choice of the in-vitro culture of oocytes and embryos according to the standard routine or with mechanical agitation (micro-vibration) until transplantation. Written informed consent was obtained from all the participating couples. Patients with unexplained infertility were stimulated for IVF/intracytoplasmic sperm injection (ICSI) with triptorelin (Decapeptyl; Ferring, Kiel, Germany) and recombinant FSH (Puregon; Organon, Oss, The Netherlands) according to the long protocol. Ovulation was induced by the administration of 10,000 IU of human chorionic gonadotrophin (HCG) (Pregnil; Organon) and oocytes were retrieved 34—36 h later and inseminated with the partner's spermatozoa through conventional IVF and ICSI techniques.

Permission was granted by the Ethical Commission of Medical Faculty of University Ulm (Germany) for the in-vitro culture of embryos under mechanical agitation.

Oocytes for the culture of pronuclear embryos were obtained from 148 informed patients aged 25—47 years (median age 36.1). Pronuclear embryos (two per patient) were cultured *in vitro* under two different conditions: group 1 (74 patients, n = 148), without mechanical agitation of the culture medium (standard routine conditions); and group 2 (74 patients, n = 148), with mechanical agitation (44 Hz delivered over 5 s once every hour and acceleration (660 mV/g at 3.3 V: X = ±1.0g, Y = ±0.7g, Z = ±0.15g)). Patients were alternately assigned to the two embryo culture groups. Only two embryos per patient were cultured, as according to German law no more than three oocytes/embryos from one patient (usually two) can be cultured *in vitro* and all cultured oocytes/embryos must be later transferred to the patient independently of the developmental rate of these embryos; also cryopreservation of embryos is forbidden. Mechanical agitation was achieved using the newly developed device. Embryo development rates were determined 18 h later. The embryos were cultured in 50 µl of culture medium (Sage, Los Angeles, CA, USA) under mineral oil (Sigma, St. Louis, MO, USA) for 3—5 days before their transfer. It should be noted that the vibration at a frequency of 44 Hz and the acceleration described above are the parameters of movement of the plate on which Petri dishes with culture medium and embryos are located. The study laboratory's observations on bovine oocytes have shown that the amplitude of vibration of embryos as well as acceleration of cells are lower than these rates with which the plate is vibrating (data not shown). This fact is due to the inertness of oocytes suspended in a liquid environment: vibration is drastically suppressed by the culture medium and is dependent on the composition and volume of this medium. Parameters of the 'real' vibration of embryos can be calculated mathematically and an investigation for such a calculation is

currently underway. Future studies should lead to optimization of the vibration parameter.

The regime of mechanical stimulation has two parameters: duration 5 s/h and frequency 44 Hz. The first parameter (5 s with 1 h interval) was chosen taking into account the data of Mizobe et al. (2010). They examined the effects of mechanical vibration on the maturation and development of pig oocytes during in-vitro maturation and/or in-vitro culture after artificial activation. It was established that the blastocyst formation rates after activation of oocytes matured with mechanical vibration for 5 s at intervals of 30–60 min or for 10 s at intervals of 60 min were higher than those of oocytes matured without mechanical vibration (27% versus 12% and 26% versus 15%, respectively). It was noted also that the blastocyst formation rate from embryos resulting from somatic cell nuclear transfer was improved by mechanically vibrating the oocytes for 5 s at intervals of 60 min during in-vitro maturation, regardless of the presence or absence of the same treatment during in-vitro culture (17% versus 9%). The results indicated that mechanical vibration enhances the cytoplasmic maturation of in-vitro-matured pig oocytes (Mizobe et al. (2010)).

The second parameter of the regime (44 Hz frequency) can be explained by the data of Schumann (1952), which describes the so-called global electromagnetic resonance with an electromagnetic spectrum between 3 and 69 Hz. It was speculated that, for Earth life, vibration of this frequency could be evaluated as 'physiological'. The current study chose the frequency of 44 Hz on technical grounds: the minimal vibration frequency of apparatus being 44 Hz.

Embryo scoring was performed every day early in the morning. The embryo quality system used to grade the day-2 and day-3 embryos was that described by Steer et al. (1992). Embryo transfer (two embryos per patient) was performed on day 3 or day 5 post-insemination. In accordance with German legislation, the embryos were transferred regardless of their developmental stage, including degenerated and developmentally arrested embryos. Pregnancy was defined as an increase in serum HCG concentration ($\geq$12 IU/ml) determined 13–15 days after embryo transfer. Clinical pregnancy was recorded when the fetal sac was visualized on ultrasound in gestational weeks 7–8.

Results were expressed as means ± SD and percentages. Means for the groups were compared by analysis of variance while the chi-squared test was used to compare proportions. The level of significance was set at $P < 0.05$.

The mean number of oocytes retrieved per patient was 7.0 ± 3.7. The rates of pronuclei formation were similar in the static (73 ± 3.4%) and dynamic (76 ± 2.1%) in-vitro culture systems. Under the dynamic culture conditions, a significantly higher percentage of excellent (grade A) and good (grade B) quality embryos at the 4–6 blastomere stage was observed compared with the static culture system (90.1 ± 1.7% versus 77.9 ± 4.4%, $P < 0.05$). Also under the dynamic culture conditions, the percentage of embryos at different blastocyst stages was 10% higher than that recorded for the static culture system (14.1 ± 2.8 versus 4.5 ± 1.7, $P < 0.05$).

It was concluded that in-vitro culture under dynamic conditions resulted in a significantly higher pregnancy rate regardless of the day of embryo transfer. Compared with the static culture system, the pregnancy rate after the transfer of day-3 embryos was 28% higher (78.4 ± 3.2% versus 50.1 ± 4.9%, $P < 0.05$) and in response to the transfer of day-5 embryos was 39% higher (72.2 ± 1.5% versus 33.2 ± 2.4%, $P < 0.01$).

Pulsative mechanical micro-vibration: mechanism of positive effect

What is known about micro-vibration in general? Vibration is a natural phenomenon that refers to mechanical oscillations about an equilibrium point. Since life began, the Earth has subjected all living things to a natural pulsation frequency. This natural phenomenon was predicted in 1952 (Schumann, 1952) and named the global electromagnetic resonance phenomenon or Schumann resonances. Schumann resonances are quasi-standing electromagnetic waves that exist in the Earth's 'electromagnetic' cavity (the space between the surface of the Earth and the ionosphere). Schumann resonances are the principal background in the electromagnetic spectrum between 3 and 69 Hz and appear as distinct peaks at extremely low frequencies of around 7.83 (strongest), 14, 21, 27, 39 and 45 (weakest) Hz. In daily life, this vibration could be 'desirable' (for example musical instruments), but more often is undesirable (wasting energy and creating unwanted sound – noise).

The stimulating effect of vibration on living systems is well known and plays a relevant role in mechanical transduction, which is essential for the survival of both cells and higher organisms. According to Sachs (1988), mechanoreceptors at the cell level provide the feedback for avoidance reactions in free-swimming protozoans and for the gravitational and tactile reactions that occur in plants. Mechanotransduction mechanisms are probably essential for regulating cell volume, proliferation and metabolic activity and are mediated by different intracellular pathways (Rosenberg, 2003). The known properties of mechanotransducers can be accounted for by ion channels, whose gating is controlled by membrane potentials.

Recent in-vivo and in-vitro studies have shown that some dynamic loads, such as mechanical vibration, have a beneficial effect. Thus, loading enhances the tendon fibroblasts of embryonic chicks, promotes the healing of fractures in rabbits and rats, increases the concentration of insulin-like growth factor I (IGF-I) in the tendons and peripheral nerves of rats, and relieves neurogenic or musculoskeletal pain in humans (Bayliss et al., 1986; Hansson and Dahlin, 1988; Inerot et al., 1991; Jancovich, 1972; Lurdeberg, 1984; Ryusuke and Horoshi, 1992; Sekiya, 2000; Yasuo and Joseph, 1989). While the mechanisms of action of these effects remain unclear, they likely differ with different modes of vibration (Jancovich, 1972; Yasuo and Joseph, 1989; Ryusuke and Horoshi, 1992).

In mice, short exposures to low-level (90 Hz, ± 0.2*g*) whole-body vibrations can limit adipogenesis while stimulating osteoblastogenesis. For instance, a 6-week vibratory intervention was found to increase the overall marrow-based stem cell population by 37% and the number of mesenchymal stem cells by 46% (Luu et al., 2009). After 14 weeks, visceral adipose tissue formation was suppressed by 28%, whereas the trabecular bone volume fraction in the

tibia was increased by 11%. As bone and muscle cells originate from the same pool of progenitor cells, it is entirely conceivable that mechanically altered bone marrow cell populations link the changes between different tissues within the musculoskeletal system. These authors noted that the anabolic and anti-catabolic effects of whole-body vibrations on the skeleton are unlikely to require muscular activity to become effective. Even high-frequency signals that induce bone matrix deformations of far less than 5 microstrains can promote bone formation in the absence of muscular activity, and these mechanical interventions are both safe and effective (Judex and Rubin, 2010; Judex et al., 2007; Usui et al., 1989). In sports medicine, the properties of low-level mechanical vibration used as a massage tool and/or for training purposes are well known. The observed positive effects of whole-body vibrations are dependent on several neural facilitatory and inhibitory mechanisms and generate global neuromuscular, metabolic and hormonal responses (Issurin, 2005; Roll et al., 1989).

Mechanical vibration applied to somatic cells cultured *in vitro* activates their proliferation and secretory properties. Observations include: (i) the enhanced proliferation of bovine articular chondrocytes (Kaupp and Waldman, 2008); (ii) the time-dependent augmentation of DNA synthesis and also the promotion of proteoglycan synthesis during long-term culture in response to periodic vibration at a frequency of 300 Hz (Liu et al., 2001); and (iii) increased interleukin-8 release in human bronchial epithelial cells (Puig et al., 2005). In addition, vocal fold-like vibrational stimuli have been found to influence the expression of several key matrix and matrix-related genes, enhance the secretion of the profibrotic cytokine transforming growth factor β1, increase the accumulation of the extracellular matrix proteins, fibronectin and collagen type 1, stimulate vocal mucosa-like matrix expression by hydrogel-encapsulated fibroblasts and enhance construct stiffness compared with non-stimulated controls (Kutty and Webb, 2010; Wolchok et al., 2009). The latter authors suggest that mechanical vibration is a critical epigenetic factor regulating vocal fold extracellular matrix (ECM) and propose that rapid restoration of the phonatory microenvironment may provide a basis for reducing vocal scarring, restoring native matrix composition and improving vocal quality (Kutty and Webb, 2010). Further observations include moderate frequency vibrations (300 Hz, ± 1.4*g*), which facilitate the biosynthetic response of chondrocytes *in vitro* (Liu et al., 2001); and 25 Hz vibrations increase major matrix proteins in bone tissue and regulate the expression of vesicular endothelial growth factor (VEGF) variants, indicating that appropriate combined loading has the potential to functionalize cellularized bone-like constructs (Dumas et al., 2009). It is also known that VEGF is a potent mitogen in endothelial cells (Ferrara et al., 2003) and plays a critical role in the induction and regulation of angiogenesis in physiological processes such as embryonic development and the menstrual cycle as well as in pathological conditions (e.g., tumour growth and atherosclerosis) (Ferrara, 2005; Gu and Adair, 1997; Pages and Pouyssegur, 2005).

A significant increase in VEGF immediately after exercise has been linked to exogenously induced vibrations of a frequency of 30 Hz (Suhr et al., 2007). These results support the contention that mechanical stimuli differentially influence factors involved in the induction of angiogenesis. Intercellular communication enables cells to co-ordinate their physiological activity and establish cell co-operation. This confers on cell systems the ability to respond uniformly to a localized stimulus as an important response in cell behaviour and differentiation (Caveney, 1985; Loewenstein, 1981; Spray and Bennett, 1985; Fraser et al., 1987). The results of the current study directly or indirectly support the findings of all these investigations.

Also, results of the current investigations are mostly consistent with the recent results of Heo et al. (2010) and Matsuura et al. (2010). Thus, the mechanical stimulation of embryos during in-vitro culture with 5 s intervals of 44 Hz/h observed herein significantly increased the developmental competence and quality of human embryos, compared with the standard static culture system, but did not influence the fertilization rate of the injected oocytes.

Pulsative mechanical micro-vibration stimulates intracellular signals

Thus, the main characteristic shared by the three types of in-vitro culture methods discussed here (pulsatile dynamic microfunnel culture, tilting embryo culture and mechanical micro-vibration) is mechanical stimulation, which seemingly induces cell-to-cell communication. It is proposed that further beneficial effects on embryo development of all these techniques are refreshing of the medium surrounding the embryo and perfusion (Heo et al., 2010), achieved by tilting or mechanical vibration (Matsuura et al., 2010; Mizobe et al., 2010), resulting in mixing of the medium and reducing concentrations of toxic embryo metabolites.

The mechanism whereby extracellular mechanical signals are transformed into intracellular signals is not fully understood. However, cell-surface receptors for matrix molecules are likely to be involved in these response processes (Karin et al., 1995). In effect, the multifactorial influence of mechanical vibration may be explained by activation of intracellular cell-to-cell communication. Thus, it has been proposed that intercellular communication enables cells to co-ordinate their physiological activity and establish cell co-operation. This gives cell systems the ability to respond uniformly to a localized stimulus and this response is important in cell differentiation and behaviour (Caveney, 1985; Fraser et al., 1987; Loewenstein, 1981; Spray and Bennett, 1985). Research by Dirksen's group (Sanderson et al., 1990) has clearly shown that mechanical stimulation of ciliated epithelial cells in culture induces a wave of increasing Ca^{2+} that spreads from the stimulated cell to neighbouring cells. In the absence of extracellular Ca^{2+}, these mechanically stimulated cells showed no change or a decrease in $[Ca^{2+}]_i$, whereas $[Ca^{2+}]$ increased in neighbouring cells. Additionally, iontophoretic injection of inositol 1,4,5-trisphosphate (IP_3) in treated epithelial cells in culture evoked a communicated Ca^{2+} response that was similar to that produced by mechanical stimulation. These data allowed these authors to postulate that IP_3 acts as a cellular

messenger that mediates communication through gap junctions between ciliated epithelial cells.

Conclusion

The in-vitro culture of human embryos in a medium subjected to regular short intervals of mechanical agitation leads to increased development rates. This type of treatment attempts to mimic conditions in nature whereby oviductal fluid is mechanically agitated by the epithelial cilia. These early results will benefit from independent confirmation and the further analysis of variables.

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