Advances in embryo culture platforms: novel approaches to improve preimplantation embryo development through modifications of the microenvironment

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BACKGROUND: The majority of research aimed at improving embryo development in vitro has focused on manipulation of the chemical environment, examining details such as energy substrate composition and impact of various growth factors or other supplements. In comparison, relatively little work has been done examining the physical requirements of preimplantation embryos and the role culture platforms or devices can play in influencing embryo development.
**Methods:** Electronic searches were performed using keywords centered on embryo culture techniques using PUBMED through June 2010 and references were searched for additional research articles.

**Results:** Various approaches to in vitro embryo culture that involve manipulations of the physical culture environment are emerging. Novel culture platforms being developed examine issues such as media volume and embryo spacing. Furthermore, methods to permit dynamic embryo culture with fluid flow and embryo movement are now available, and novel culture surfaces are being tested.

**Conclusions:** Although several factors remain to be studied to optimize efficiency, manipulations of the embryo culture microenvironment through novel culture devices may offer a means to improve embryo development in vitro. Reduced volume systems that reduce embryo spacing, such as the well-of-the-well approach, appear beneficial, although more work is needed to verify the source of their true benefit in human embryos. Emerging microfluidic technology appears to be a promising approach. However, along with the work on specialized culture surfaces, more information is required to determine the impact on human embryo development.

**Key words:** blastocyst / microfluidic / surface coating / culture device / embryo culture

**Introduction**

The introduction of sequential culture media has been one approach employed in an attempt to improve in vitro embryo culture, designed to mimic the changing chemical environment between the fallopian tube and uterus experienced by the embryo in vivo, thereby meeting its changing nutritional needs (Gardner and Lane, 1998). Additionally, improved monoculture systems have also resulted in improved embryo development (BIGGERS et al., 2005; BIGGERS and SUMMERS, 2008). However, embryo progression through the female reproductive tract not only results in fluid exchange around the embryo, but also provides gentle mechanical stimulation (FAUCI and DILLON, 2006), which may affect embryo development. In comparison to the immense amount of data regarding the influence of chemical conditions on improvement of in vitro embryo culture, including salts, amino acids, energy substrates and various other additives, physical requirements for embryos development in vitro have been largely neglected. Fortunately, emerging technology has allowed for the development and examination of various novel culture platforms to explore these physical requirements in the hope of optimizing embryo development in vitro.

**Static culture platforms**

Historically, gametes and embryos have been cultured on/in inert plastic vessels, ranging from test tubes to various configurations of Petri dishes. For the purpose of this review, these approaches are considered ‘static’ culture platforms, in that they do not employ active means to agitate or stimulate embryo or media movement. Each of these rudimentary devices carries some benefit based on a particular laboratory’s protocol. Variables considered often include physical parameters such as volume of media or density of embryos and have been elegantly reviewed (REED, 2006). To summarize, in various animal models, increased embryo density improves development, possibly through secretion of trophic autocrine/paracrine factors. Indeed, embryos secrete various factors (BORMANN et al., 2006; KATZ-JAFFE et al., 2006) and these growth factors may influence embryo development (RICHTER, 2008). Supporting this notion, platforms utilizing smaller volumes of media with a confined surface area have been found to be beneficial compared with larger vessels (LANE and GARDNER, 1992; VAJTA et al., 2000; THOAS et al., 2003; ALI, 2004), possibly acting as a mechanism to concentrate secreted compounds. Thus, manipulation of the physical culture environment by altering embryo spacing can potentially manipulate the chemical environment and impact embryo development. In agreement with these findings, decreased spacing of embryos is also beneficial. Studies in pig and bovine models have shown that when adhering embryos to the bottom of culture dishes at varying distances from one another, or using varying polyester mesh to control distance, embryos in closer proximity to each other had improved development (STOKES et al., 2005; GOPICHANDRAN and LEES, 2006; SOMFAI et al., 2010). Studies citing a benefit of group culture are largely from the mouse (Canseco et al., 1992; Lane and Gardner, 1992), a litter-bearing species; although references demonstrating benefit of group culture can be found in bovine and sheep as well (GARDNER et al., 1994; DONNAY et al., 1997; O’DOHERTY et al., 1997; KHURANA and NIEHANN, 2000; FUJITA et al., 2006; GOOVAERTS et al., 2009). This may be insightful as to the role of autocrine versus paracrine effects. Another important variable in these studies may be the quality of companion embryos (SPINDLER and WILD, 2002; SPINDLER et al., 2006). Additionally, this group effect (Moessner and Dodson, 1995; Almagor et al., 1996; Ebner et al., 2010; Rebollar-Lazaro and Matson, 2010) or spacing effect may or may not be apparent in human embryos, suggesting that well-designed studies remain to be performed.

Although current common practice is to culture groups of embryos in smaller volumes, this approach requires careful attention to media properties, as shifts in pH and osmolality are more likely. Additionally, it should be mentioned that individual embryo culture is feasible, and use of larger volumes of media still produce high-quality embryos with a corresponding high pregnancy rate in many laboratories. Regardless of which of these approaches is utilized, a commonality between protocols is that embryos are generally cultured in a static environment on inert plasticware, with limited cell-surface contact, which may be neglecting their physical requirements. Fortunately, new static culture approaches are being examined which may offer a means of improving current culture techniques, in part, by further manipulating physical parameters (Fig. 1).

**Specialized microdrop dishes**

Novel devices are now being developed that alter the physical culture environment to manipulate embryo spacing and take advantage of any autocrine/paracrine effect. One abstract reports that culture of
mouse embryos in the commercially available ‘embryo GPS dish’ showed better development compared with embryos grown in similarly sized microdrops on a flat Petri dish, yielding greater rates of morula formation and compaction on Day 3 (Rieger et al., 2007). This specialized dish consists of small round bottom wells distributed in a traditional Petri dish, which promote gathering of embryos in close proximity (Fig. 1). This design allows for rapid identification and recovery of cells, also alleviating problems with traditional microdrops, which include drops shifting or coalescing. An additional modification of the GPS dish, known as the Embryo Corral, further divides each well into four sections using vertical posts, thereby allowing individual embryos to be cultured in each section for tracking purposes, while allowing embryos to share a common media reservoir.

**Ultramicrodrops**

A variation of the microdrop, the ultramicrodrop, utilizes 1.5–2.0 µl of media to continuously culture groups of 7–9 embryos for 2–3 days in the hopes of confining embryos to a very small area and taking advantage of beneficial autocrine/paracrine factors (Ali et al., 2000; Ali, 2004). Using this approach, the authors were able to obtain high pregnancy rates, and though involving small numbers, were able to show improved human embryo development and quality using sibling embryos in a prospective manner compared with larger 20 µl microdrops. Dealing with such small volumes of media does require careful attention and presents potential problems if not performed correctly. Handling such small volumes of liquid is technically challenging and can be highly variable. Issues include potential for rapid evaporation and damaging osmolality increases (Swain et al., 2010), potential toxicity due to high embryo density or embryo loss if media exchange is not performed properly.

**Submicroliter platforms**

To explore the possibility of submicroliter culture of mouse embryos, a culture ‘chip’ composed of polydimethylsiloxane (PDMS) was produced consisting of small vertical channels, where 2-cell embryos were placed into a larger reservoir and allowed to fall down into isolated channels with 100 nl volumes and observed microscopically (Melin et al., 2009). Rates of blastocyst development were comparable with those from embryos cultured in 20 µl microdrops (81.8 versus 83.3%), and were significantly greater than embryos grown in 5 µl microdrops. Thus, this novel system appears to allow embryos to benefit from greatly reduced culture volume and reduced spacing, while avoiding some issues associated with small microdrop volumes, but does include potential detrimental issues of its own, including embryo recovery.
Microwells

Additional variations on the microdrop method of embryo culture have emerged. Although these could be also classified under the above heading of specialized microdrop dishes, these approaches utilize a very small area, and are commonly referred to as microwells. Microwells aim to create a small microenvironment for individual or small groups of embryos, while allowing them to share a larger common culture media reservoir (Fig. 1). These approaches avoid pitfalls associated with traditional microdrop displacement or merging, but also offer a means of further altering the physical environment experienced by the embryo by potentially increasing surface area contact to three plains of the developing embryo, although this contact is limited to interaction with the zona pellucida. What effect this increased surface contact has is unknown.

Microwell approaches are often termed the well-of-the-well (WOW) approach, as first described by Vajta (Vajta et al., 2000, 2008). The WOW approach entails using small impressions, or microwells, placed into the bottom of a larger 35 mm or 4-well plastic dish with a variety of methods. These microwells can be made to varying sizes and arranged in a variety of configurations, so that either single or small groups of embryos can be isolated in individual microwells yet share the same overlying media for a group effect (Table I).

Utilizing the WOW approach with wells of an approximate width and depth of 250 x 200 μm overlaid with media and oil has resulted in improved development of bovine, mouse and pig and human embryos in comparison to culture in varying sizes of microdrops (Vajta et al., 2000, 2008), and the improvement appears to be independent on the number of WOWs utilized (Vajta et al., 2000). However, at least one study with pig embryos does indicate that size of the WOW housing the embryo may be important, as 1000 μm wide wells yielded greater blastocyst development than 500 μm wells (Taka et al., 2005). A similar WOW approach also resulted in differing gene expression levels in bovine embryos compared with microdrop cultured counterparts, perhaps offering further insight into potential benefits (Hoelker et al., 2009).

More recently, a WOW system was fabricated in polystyrene using injection molding to allow time-lapse photography of developing bovine embryos. Wells were 287 μm wide x 168 μm deep, with 25 wells (5 x 5 configuration), each holding individual embryos, overlaid with 125 μl of media. Although no differences in blastocyst development (37.2 versus 36.0%) or cell number (112 versus 103) were observed in WOWs compared with microdrop controls, WOW cultured embryos had lower amounts of apoptosis (9.0 versus 13.5) and oxygen consumption, closer to in vivo derived counterparts (Sugimura et al., 2010). Ultimately, these WOW culture embryos resulted in significantly higher pregnancy rates at 60 days following transfer (51.7 versus 21.9%).

A similar approach has also been pursued using WOWs made of PDMS. Microwells were ~346 μm wide x 200 μm deep and a series of 25 wells (5 x 5 configuration) were overlaid with ~100 μl culture media for individual embryo culture (Akagi et al., 2010). Although blastocyst development rates were low, and there was no added benefit over use of equal sized microdrops (17.5 versus 16.7%, respectively), this manufacturing approach does offer an alternative means of making the microwell approach commercially and more widely available.

An alternate approach to creating microwells in the bottom of polystyrene dishes or PDMS entails using a micro-well-insert consisting of several rows of tiny culture wells composed of PDMS. This approach has been developed to allow the culture of multiple individual embryos (Krisher and Wheeler, 2010). These inserts can be adapted to adhere to the bottom of any existing traditional culture plasticware and effectively reduced the surface area and volume of media around an embryo. Importantly, these inserts can be produced with isolated wells, as well as with microfluidic channels connecting the wells to allow media flow and to take advantage of the group culture effect. These connected inserts could be useful in teasing out the impact of autocrine/paracrine compounds versus embryo spacing. Although optimal well size and media volume are still being explored, initial devices without connecting microchannels have been used to successfully culture in vitro produced pig, cow and mouse zygotes to the blastocyst stage, yielding similar results to embryos cultured in microdrops (Krisher and Wheeler, 2010). These devices provide an easy-to-use consistent alternative to previous cumbersome micro-techniques for individual embryo culture.

Although less elegant than PDMS well inserts, polyester mesh inserts have also been used to culture porcine and bovine embryos and have similarities to microwells (Booth et al., 2007; Matoba et al., 2010; Somfai et al., 2010). Use of this mesh allows for easy separation and identification of embryos, as each square of the grid houses an individual cell. This approach allows for decreased spacing of embryos and separation of cells as a specific distance, with any associated benefit. Indeed, use of a 217 μm mesh size (embryo spacing of ~177 μm) yielded increased blastocyst cell number compared with controls or embryos cultured in larger 230 or 238 μm mesh (Somfai et al., 2010). Furthermore, this approach increases potential surface area for points of embryo/zona pellucida contact and may facilitate formation of microenvironments or localized gradi- ents. Additional mesh sizes remain to be examined to delineate any additional benefit.

Microchannels

As an alternative to microwells, use of microchannels has been examined for culturing embryos. Microchannel approaches are intriguing as they offer an additional means to increase surface area with developing embryos/zona pellucida compared with culture on flat-bottomed dishes (Fig. 1). Although channel size was considerably larger than developing embryos, Raty et al. found that 2-cell mouse embryos could be cultured to the blastocyst stage within static PDMS microchannels situated on a traditional glass slide (Raty et al., 2001, 2004). These experiments demonstrate that, compared with 30 μl control microdrops, culture within microchannels containing ~500 μl of media (10 μl within the actual channel itself flanked by two reservoirs of media) resulted in significantly greater 16-cell/morula formation at 24 h (23.5 versus 4.7%), greater blastocyst formation at 48 h (17.6 versus 2.4%) and 72 h (72.9 versus 42.9%), and a greater portion of hatched blastocysts at 72 h (4.1 versus 0%) and 96 h (26.5 versus 8.8%). Using this microchannel approach, it was estimated that the effective volume of media surrounding the embryo was ~250 nl, although this would assume no mixing of the surrounding media (1 ml) (Beebe et al., 2002). A subsequent abstract utilizing a similar device by the same research group showed that
in vivo derived 4-cell porcine embryos could be cultured to blastocysts and transferred, resulting in live birth (Walters et al., 2003). However, in these experiments, no observable beneficial effects on embryo development were seen when compared with culture in control organ-well dishes.

Similarly, although not as elegant in design as the microchannels described above, culture in microliter volumes in glass capillary tubes, which are essentially microchannels, also supports mouse development from the zygote to blastocyst stages (Lane and Gardner, 1992; Thouas et al., 2003). This approach also permits embryos to be cultured vertically, which promotes increased cell contact (decreased cell spacing), but may also offer additional physical advantages. Whether vertical embryo culture is beneficial is unknown, but it has been shown that a microgravity environment is detrimental to embryo development, yielding fewer trophectoderm cells and lower pregnancy rates following transfer (Wakayama et al., 2009).

### Summary

Based largely on data from animal models, several novel culture approaches utilize decreased volumes to culture preimplantation embryos and appear to offer potential benefit to resulting embryo development. However, to date, no thorough studies have been conducted to determine if this approach truly benefits human embryos and whether potential benefit may be due to reduced culture area, reduced embryo spacing and/or potential concentration of trophic autocrine/paracrine factors. In these cases, group culture and quality of companion embryos would undoubtedly be a significant factor in the experimental design. Indeed, two recent publications indicate that group culture improves rates of human blastocyst development and quality compared with individual culture (Ebner et al., 2010; Reboliar-Lazaro and Matson, 2010). Regardless, when considering factors such as embryo recovery, commercially available specialized microdrop dishes are a user-friendly alternative to traditional microdrops that do appear to offer ease-of-use benefit. Emerging microwell approaches appear promising and are easily implemented into current laboratory practice when commercial devices become widely available, although more research is needed to determine if increased surface interaction may be an added source of benefit, as well as what well size, well number and overlying media volume may be optimal. It should also be pointed out that utilization of small volumes, such as ultramicrodrop and microchannel approaches, has potential shortcomings. Greater attention to detail is required with these approaches, as conditions using low media volume may be more permissive for detrimental changes to media conditions, such as osmolality (Swain et al., 2010) or pH. Furthermore, likely to a lesser extent, concern with ease of embryo recovery may exist. As future designs improve, it is hoped that these potential pitfalls can be overcome to take advantage of the immense potential offered, not only to overall embryo development, but for the potential to concentrate secreted embryonic factors and allow for more sensitive analysis of secreted molecules in the hopes of finding markers of embryo quality.

<table>
<thead>
<tr>
<th>Species</th>
<th>Well size (w × h)</th>
<th>Conditions (Test versus Con)</th>
<th>End-point (from 1-cell)</th>
<th>Outcome (Test versus Con)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>250 × 200 μm</td>
<td>I embryo/WOW in 500 μl; 1 embryo/20 μl μdrop (SOFaa media)</td>
<td>Blast at 168 h</td>
<td>60 versus 34% (P &lt; 0.05)</td>
<td>Vajta et al. (2000)</td>
</tr>
<tr>
<td>Bovine</td>
<td>700 × 700 μm</td>
<td>I embryo/WOW (16 total) in 500 μl; 16 embryos/500 μl μdrop (CR1aa media)</td>
<td>Blast at 192 h; blast cell#; apoptosis</td>
<td>31 versus 22% (P &lt; 0.05); 99.6 versus 99.3 (NS); 2.8 versus 2.6% (NS)</td>
<td>Hoelker et al. (2009)</td>
</tr>
<tr>
<td>Bovine</td>
<td>287 × 168 μm</td>
<td>I embryo/WOW (25 total) in 125 μl; 25 embryos/125 μl μdrop (CR1aa media)</td>
<td>Blast at 168 h; blast cell#; apoptosis; pregnancy (30 days)</td>
<td>37 versus 36% (NS); 11.1.5 versus 102.7 (NS); 9.0 versus 13.5% (P &lt; 0.05); 51.7 versus 25% (P &lt; 0.05)</td>
<td>Sugimura et al. (2010)</td>
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<tr>
<td>Bovine</td>
<td>346 × 200 μm</td>
<td>I embryo/WOW (20 total) in 100 μl; 20 embryo/100 μl μdrop (IVD101 media)</td>
<td>Blast at 192 h; blast cell#</td>
<td>17 versus 18% (NS); 81.4 versus 84.5% (NS)</td>
<td>Akagi et al. (2010)</td>
</tr>
<tr>
<td>Bovine</td>
<td>1000 × 700 μm</td>
<td>I embryo/WOW (20 total) in 100 μl; 20 embryos/100 μl μdrop (SOF media)</td>
<td>Blast at 168 h</td>
<td>37 versus 30% (NS)</td>
<td>Matoba et al. (2010)</td>
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<tr>
<td>Porcine</td>
<td>1000 × 300 μm</td>
<td>4–5 embryo/WOW (3 total) in 500 μl; 12–15 embryos/30 μl μdrop (PZM3 media)</td>
<td>Blast at 192 h; blast cell#</td>
<td>25 versus 13% (P &lt; 0.05); 36 versus 37 (NS)</td>
<td>Taka et al. (2005)</td>
</tr>
<tr>
<td>Murine</td>
<td>250 × 200 μm</td>
<td>I embryo/WOW (5 total) in 400 μl; 1 embryo/35 μl μdrop (CZB media)</td>
<td>Exp blast at 144 h</td>
<td>80 versus 40% (P &lt; 0.05)</td>
<td>Vajta et al. (2008)</td>
</tr>
<tr>
<td>Human</td>
<td>250 × 200 μm</td>
<td>I embryo/WOW (5 total) in 600 μl; 1 embryo/80 μl μdrop (Sage media)</td>
<td>Blast at 120 h</td>
<td>55 versus 37% (P &lt; 0.05)</td>
<td>Vajta et al. (2008)</td>
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</table>

### Table 1

Summary of studies using various well-in-the-well (WOW) devices.

In addition to species specificity, the size of wells and amounts of media are important variables when considering success of this static embryo culture approach.
Dynamic culture platforms

Although static embryo culture has been the predominate method employed to date, continuing research emerges examining the effect of dynamic culture conditions on embryo development in vitro. That being said, it should be mentioned that no culture platform is completely static, as convection currents and gentle movement of dishes during routine observation agitate the media. However, in the context of this review, ‘dynamic’ culture platform refers to culture devices purposely engineered to stimulate controlled media flow/movement.

As theoretically promising as dynamic culture appears, one of the limitations of these systems is their relative complexity in comparison to static culture devices. Several criteria and considerations must be met before any dynamic culture system receives widespread implementation, and these include ease-of-use and lab compatibility (Table II). Additionally, biocompatibility is paramount. When dealing with dynamic systems, a specific concern exists with sheer stress. Indeed, embryos can sense sheer stress and excessive mechanical stimulation can damage cells (Xie et al., 2006, 2007). Sheer stress over 1.2 dyn/cm² results embryo degeneration within 12 h (Xie et al., 2006). Movement or flow rate as well as pattern of movement (motion path, continuous or periodic, etc.) are obvious factors influencing sheer stress. However, volume of media and oil may also influence forces exerted upon the embryo during various movement schemes, and factors such as friction or impact against platform edges may also be important variables. Therefore, any dynamic culture device needs to consider these factors in order to gain widespread implementation (Table III).

Furthermore, determination of causality for observed benefits from dynamic culture may aid in simplification of devices to aid in this endeavor (Table IV). Possible explanations for benefits of dynamic culture systems include removal of harmful byproducts. Indeed, degenerating embryos appear to have a negative impact on ‘normal’ appearing companion embryos (Spindler and Wildt, 2002; Spindler et al., 2006). Additionally, degradation of media components can also compromise embryo development. As an example, amino acid breakdown in culture at 37°C into ammonia may negatively impact embryos and resulting fetuses (Gardner and Lane, 1993; Lane and Gardner, 1994, 2003; Lane et al., 2001; Zander et al., 2006), although not all agree that the levels of ammonia needed to observe these effects can be reached from commercial culture media (Biggers et al., 2004). However, regular replacement of media to remove harmful factors has not proven beneficial, possibly due to added stresses from repeated manipulations outside the incubator. Continuous culture of embryos in ultramicrodrops was superior compared with regular replacement of media up to Day 3 of development (Ali, 2004). Additionally, in bovine, continuous culture in microdrops for 138 or 186 h yielded superior blastocyst development compared with replenishment of media at 48 h intervals, despite higher concentrations of ammonia build-up (Fukui et al., 1996).

It is also known that gradients exist in culture media, due to embryo secretions or depletion of media components. Gradients of potassium, calcium and oxygen have been measured around mouse embryos (Trimarchi et al., 2000a, b) and dynamic culture platforms may disrupt these gradients, providing a more homogenous environment thereby improving the culture conditions. Alternatively, although no data exist to confirm the hypothesis, gentle stimulation of embryos could activate beneficial mechanoreceptors or signaling pathways to promote embryo growth. It is known that embryos can sense sheer stress and activate various protein kinases (Xie et al., 2006, 2007). It is feasible that some intermediate levels of pathway activation have embryo-trophic effects.

Although theoretical benefits of dynamic culture are now evident, any actual advantage likely relies on a host of factors. Importantly, not all dynamic culture devices can incorporate all the above-mentioned characteristics and may not necessary benefit embryo growth. Several approaches to generate dynamic embryo culture have been examined and are discussed below (Fig. 2).
**Shaking/rotation**

A few publications have examined the impact of physical stimulation on mouse fertilization by utilizing a laboratory shaker system within the confines of a traditional incubator (Hoppe and Pitts, 1973). Using 0.5 ml of media with 2 ml oil overlay, cells were agitated on an orbital shaker at 60 rev/min. Different volumes of media (0.2, 1.0 ml) and different times of agitation (1, 3, 5, 8, 11 h) did not appear to have a significant effect. Although the authors did not compare this system with a static system, it did show that ova could be fertilized in a dynamic environment with movement. A similar approach has also been utilized with ovarian tissue culture, using 30 ml volumes and 75 rev/min (Itachenko et al., 2006). Additional studies exist using a heated, agitated stage, with a petri dish of microdrops covered in oil resting on top to culture mouse or rat embryos (Zeilmaker, 1973, Cohen et al., 1981). One factor to consider is that the use of a mechanical shaker in a humidified incubator can be problematic due to fumes or electrical failure. Unpublished work in the author's laboratory suggests that rates of rotation can significantly impact embryo development, with high rates of orbital movement (60 rev/min) being detrimental to mouse embryo development. One-cell frozen/thawed mouse embryos (n = 20) showed significantly lower blastocyst development at 96 h in 50 μl drops following rotation compared with static counterparts (5 versus 90%). This negative impact of orbital movement may also be influenced by the shape of the culture environment, as slower orbital movement using a flat PDMS culture surface was beneficial for fresh mouse embryos, but became detrimental when embryos were cultured in microwells (Oakes et al., 2009). Data presented in abstract form indicated that orbital rotation on flat surfaces yielded significantly greater rates of blastocyst development (98.5%) compared with static culture (86.3%). However, when cultured in dynamic microwells, development significantly decreased (61.6%) (Oakes et al., 2009). Furthermore, additional considerations include the possible need for specialized dishes to avoid coalescing or combining of microdrops/embryos due to agitation. Additional variables such as type of movement (circular or linear) and volume of media/type of dish all can influence the type of forces exerted upon the embryo and remain to be explored.

**Tilting**

A more recent publication has examined the impact of embryo and media movement on both mouse and human embryo development using a tilting embryo culture system (TECS) (Matsuura et al., 2010). Consisting of a special motorized tilting platform placed inside an incubator, traditional culture dishes can be placed on the device and tilted to various angles at various rates to try to mimic similar forces experienced by embryos in vivo as they move through the reproductive tract. Using this approach, dishes were tilted to 20° at 1°/s and held for 1 min, yielding an estimated sheer force of ~0.7 dyn/mm². Under these suboptimal conditions in 500 μl volumes, it was shown that mouse blastocyst development from the 2-cell stage and cell number significantly improved when cultured in TECS (42%, 77 ± 2) compared with static culture (27%, 66 ± 4). Culture of 10 embryos in 50 μl microdrops showed no difference between treatments, while culture of 5 embryos in 50 μl microdrops also showed a significant improvement in blastocyst development with TECS compared with static controls (59 versus 46%). Thus, TECS appears to benefit mouse embryo development under suboptimal conditions. Additionally, the device was used to successfully culture frozen/thawed human embryos, and yielded higher blastocyst cell number.

Subsequently, an additional tilting culture system was explored, examining the impact on bovine embryo development when using straight microchannels (200 μm x 50 mm x 200 μm, w x l x h) or microchannels with a 150–160 μm constriction. Embryos were tilted 10° over 1 min. Although no difference in blastocyst formation was observed or reported, the influence of the tilting system alone was not examined, the authors suggest that combining an embryo tilting system with a 169 μm constricted microchannels may

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**Figure 2** Diagrams and photographs of two promising dynamic culture approaches. (A, A', A'') A TECS is compatible with current culture dishes and provides a relatively simple way for provide gentle physical stimulation to embryos, as they roll and media is agitated during the defined tilting angle/time. (B, B', B'') Dynamic microfunnel culture with regulated media flow driven by piezo actuated Braille pins allows for programming of media flow rates and patterns, gently moving and agitating media around the embryo.
offer a means of improving bovine embryo cleavage, yielding higher rates of 8-cell development after 44 h or culture compared with straight channels (56.7 versus 23.9%) (Kim et al., 2009). Future work remains to be done examining optimization of tilting rates and angles, but offers an easy-to-implement alternative to current static culture systems.

Vibration

Dramatic movement of media or embryos may not be required to convey beneficial effects on dynamic culture. A simple vibrating culture platform, on which culture dishes are placed, has been used to mature porcine oocytes and culture resulting parthogenetically activated embryos. Although no significant effect was observed following vibration of activated embryos on blastocyst development, vibration of oocytes appeared to improve oocyte developmental competence. Mechanical vibration of oocytes at 30 or 60 min intervals for 5 s resulted in improved cytoplasmic maturation, as evidenced by improved blastocysts development compared with 90 min intervals or no vibration (25.7 versus 28.1 versus 16.0 versus 12.3%, respectively) (Mizobe et al., 2010). Additionally, vibration of oocytes for durations of 5 or 10 s yielded greater blastocyst formation compared with vibrating for 30 s (27.1 versus 25.8 versus 9.0%, respectively), indicating that extended or constant vibration may be detrimental. Future studies examining vibration frequency, as well as more in-depth studies on vibration paradigms in other species, may prove informative.

Controlled fluid flow

Perfusion devices offer the ability to replenish culture media and remove harmful byproducts, which is not possible with simple agitation/tilting devices described here. Furthermore, the true advantage of these fluid-flow systems lie in their ability to automate media flow and allow replenishment of media in a seamless fashion, without manipulations outside the incubator and associated stresses, which may be a contributing factor during manual media renewal (Fukui et al., 1996). Additionally, rather than simply relying on a two-step culture system, where embryos are rapidly exposed to a different media and potentially stressing the cells, controlled flow devices can supply an endless graduate gradient of media. Furthermore, in-line diagnostics can be implemented on perfusion platforms, where small amounts of media can be directed over various nanosensors or probes to monitor things such as pH (Baldini et al., 2007), temperature fluctuations (Chang et al., 2006; Lucchetta et al., 2006), media flow rate (Lien and Vollmer, 2007) and volume of the cell to indicate shifts in media osmolality (Ateya et al., 2005). Toward this end, sensors have been developed to measure real-time changes in levels of reactive oxygen species (ROS) on microfluidic devices (Amatore et al., 2006, 2007), embryo oxygen consumption (O’Donovan et al., 2006) or embryo secretions (Urbanski et al., 2008). One could then envision a culture system where specific media and supplements are supplied to the embryo based on what the sensors detect or what the embryo needs, thereby effectively creating an embryo-specific ‘smart’ culture environment, rather than expecting all embryos to respond favorably to a single mono- or sequential culture media.

Although the approach of dynamic media flow in embryo culture is not new, prior attempts at perfusion systems on the macro-scale for a variety of species have not been well described in the literature, and have proven inefficient and subsequently not been implemented on a large scale (Pruitt et al., 1991; Goverde et al., 1994; Lim et al., 1997). Fortunately, the unique nature of microfluidic platforms allows alternate approaches to accomplish media movement that are more amenable for wide-spread use. These methods are often dictated by constraints of platform design, which is dependent upon whether perfusion systems are recirculating or non-recirculating. Furthermore, the ability of perfusion systems to operate over long periods with minimal manipulation is essential. In adherent cell systems, perfusion devices have been operated successfully for over 1 week (Chung et al., 2005; Hung et al., 2005; Villa-Diaz et al., 2009). Various approaches to obtain media perfusion for embryo culture are discussed below. Additionally, although beyond the scope of this review, several other methods to promote media flow in microfluidic devices exist and remain to be studied in relation to embryo culture (Zhang et al., 2007; Pennathur, 2008).

Gravity

Gravity can be used to promote fluid flow via hydrostatic pressure formed from varying heights of media columns. This approach was applied with microchannel devices for use with embryos and sperm (Cho et al., 2003; Schuster et al., 2003; Clark et al., 2005; Suh et al., 2006; Schulte et al., 2007; Shibata et al., 2007). While the simplicity of the approach is advantageous, it is difficult to regulate flow rate or volume changes, especially over time when height of media columns diminishes.

Syringes

External syringes have been used to manually apply pressure to input/output ports to cause media flow through microchannel devices (Davis et al., 2000). However, lack of precision in this approach is problematic. As an improvement, Hickman et al. examined mouse embryo development in microchannels with media flow controlled via a syringe infusion pump (Hickman et al., 2002). Flow rates examined in this study (0.1 and 0.5 μl/h) did not enhance development compared with static culture. In fact, a flow rate of 0.5 μl/h resulted in significantly lower development of 2-cell mouse embryos to morula and blastocyst stages, while producing higher numbers of abnormal embryos compared with controls. Thus, flow rate and manner of flow delivery may be important variables. Although more precise and feasible for use over time than manual syringes, required external tubing and machinery is problematic for use within a closed incubator environment.

Piezo-electric actuators

Data by Cabrera and coworkers were the first report indicating that 1-cell mouse embryos could be cultured efficiently within microfluidic devices offering controlled media flow (Cabrera et al., 2006; Heo et al., 2010). Embryos were placed into a PDMS funnel reservoir, which allowed easy loading/unloading and visualization, while medium was added and removed via a microfluidic channel connected to the bottom of the funnel via actions of a Braille actuator. Braille pins were raised and lowered in a controlled fashion to depress the bottom of the microfluidic channel to create pulsatile media flow through the bottom of the funnel. It was demonstrated that regardless of media flow pattern (back and forth versus flow-through) or speed (fast
versus slow), 1-cell mouse embryos cultured in dynamic devices showed greater hatching of blastocysts and significantly higher cell number than static controls, yielding numbers similar to those obtained from in vivo derived blastocysts (Fig. 3).

Bormann et al. subsequently presented preliminary abstract data validating beneficial effects of Braille-driven media flow in a microfluidic device on murine and bovine embryo development (Bormann et al., 2007a, b). Greater number of mouse embryos reached morula stage at 48 h, blastocyst at 72 h and hatched blastocyst at 96 h compared with control static chips, while significantly more bovine embryos reached the blastocyst stage at 144 h in microfluidic devices compared with control static devices. Follow-up experiments indicated that beneficial effects of embryo culture in the dynamic culture device are additive and require a minimum 48 h of culture at the beginning or end of 96 h culture periods (Bormann et al., 2007a, b; Heo et al., 2010). The developmental rate observed was proportional to the duration of dynamic culture regardless of the stage of embryo development. Importantly, more recent studies indicate that not only is there a benefit on preimplantation embryo development, but that quality of embryos cultured in a microfluidic device with media flow is superior to those grown in static systems, as evidenced by increased implantation rates, lower rates of absorption and higher ongoing pregnancy rates in mouse (Heo et al., 2010). Interestingly, three-dimensional modeling suggests that the funnel design utilized helped retain any potential localized autocrine factors, but also allowed mechanical stimulation, thus combining benefits of microdrop and microchannel approaches (Fig. 4). Clinical trials with a modified version of this original device are currently underway.

**Additional approaches**

Yet another approach to culturing embryos within microfluidic devices has employed not only dynamic media flow, but also co-culture. Mizuno et al. have adopted a ‘womb-on-a-chip’ design, where endometrial cells are grown in a lower chamber, while embryos are cultured in an upper chamber, separated from the lower by a thin membrane (Mizuno et al., 2007), thus allowing embryos to interact with secreted factors from the endometrial cells. In preliminary abstracts, authors demonstrated that mouse ova fertilized and resulting embryos cultured in these devices showed similar cleavage to 2-cell and similar blastocyst formation rates compared with 50 μl control microdrops (Mizuno et al., 2007). Furthermore, cell number was significantly higher in blastocysts fertilized/cultured in microfluidic devices. Subsequently, blastocysts obtained from microfluidic devices

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**Figure 3** Controlled pulsatile media flow through a microfunnel has been shown to be beneficial for mouse embryo development. (A) Embryos can be easily visualized and tracked at the bottom of the microfunnel. (B) Microfluidic media flow increases rates of blastocyst hatching (C) resulting cell number and (D) implantation rates.
were transferred to recipient female mice and resulted in live offspring at rates similar to embryo cultured in static microdrops. A similar co-culture approach was taken by the same group, culturing 2-cell mouse embryos to blastocyst stage on the refined OptiCell microfluidic device. OptiCell microfluidic co-culture culture yielded chromosomally normal embryos, capable of yielding live offspring (Nakamura et al., 2007). Finally, Mizuno et al. published an abstract reporting the first instance of frozen/thawed human embryos cultured within co-culture microfluidic devices (Mizuno et al., 2007). As the goal of embryo culture has been development of defined culture media, it will be interesting to see if similar studies can be performed without the use of co-culture.

**Summary**

Dynamic culture systems are an exciting area of study that could potentially revolutionize *in vitro* embryo production. A common concern among these emerging approaches is their relative complexity and requirement to operate efficiently and safely within the humidified and warmed environment of the incubator. To date, simpler approaches such as rotation or tilting devices that can be utilized with traditional culture dishes may be easier to implement in the clinical laboratory, although no commercially available devices currently exist. Microfluidic devices perhaps offer the most potential in improving *in vitro* embryo culture, as they provide a means of controlled fluid flow that allows media exchange/modification and constrictive culture area with smaller volumes, while also providing the potential for integration of bioanalytic assays. Regardless of the dynamic approach, more work is required to determine the actual cause of benefit, and clinical trial data are needed to fully determine their feasibility and efficacy with human embryos.

**Specialized surfaces**

Another important factor to consider when exploring impact of culture platform and physical requirements of developing embryos is the surface of the device housing the embryos. Various synthetic polymers have been tested for compatibility with mouse embryo development, and not all compounds are suitable (Hunter et al., 1988). This is likely due to toxicity of additives or contaminants. Currently, most traditional culture devices are polystyrene, though glass has also been used. These materials are heat-stable compounds that can withstand the temperature and humidity of the laboratory incubator without changing media characteristics. More recently, compounds such as PDMS have been used due to ease of manufacturing using techniques such as soft lithography. Of note, PDMS must be used with caution since, if used improperly, it can absorb small compounds of the media (Toepke and Beebe, 2006), leach into the media (Regehr et al., 2009), or cause detrimental osmolality shifts (Heo et al., 2007). Regardless of which compound is used, it is clear that polystyrene, glass and PDMS are far from physiologic surfaces. In a field where physiologic basis has been a driving force in formulation of some culture media, as well as culture atmosphere (low oxygen), both resulting in improved embryo development, perhaps exploration of more physiologic culture surfaces may also lend itself to improve upon current practices.

Culture environment and surfaces used for *in vitro* embryo culture represent a large departure from the landscape of the oviduct and...
utus. In contrast to the inert, uniform surfaces utilized for in vitro culture, where embryos are submerged in modified salt solutions, the reproductive tract is a moist space consisting of convoluted surfaces composed of and coated with various polyhydroxylated compounds, macromolecules and components of the extracellular matrix (ECM). Although this contrast may seem simple to understand, it is extremely important that the true impact of these physical differences be fully realized. In contemporary in vitro culture systems, the upper end of the concentration a given component (salt, substrate, etc.) can reach in culture medium is determined by solubility. This is not the case in vivo, as compounds may and do accrue on a solid surface. In other words, the wall of the reproductive tract represents a solid phase system that is not represented in current in vitro culture conditions. The significance of this fact is great. It is well known that specific reaction rates, enzyme kinetics and other cell processes can be dramatically altered if performed on a surface rather than in solution. This has largely due to availability and ordered presentation of factors to the cell, rather than random, chaotic exposures. Thus, in this sense, one can begin to appreciate why exploration of surface topography and chemistry is certainly warranted in future pursuits of improved embryo development in vitro.

Another key physical difference between in vivo and in vitro culture conditions entails interaction of the numerous polyhydroxylated compounds found at the surface of the reproductive tract, including glycoproteins, and the surrounding fluid environment. The hydroxyl groups on these compounds alter the physical properties of water, presumably via hydrogen bonding, and thus impact biological function (Pollack, 2001; Frank, 1983). It is through these bonding interactions that one obtains gels, or similar materials, which vary dramatically from the completely fluid environment in vitro. Thus, inclusion of similar compounds in vitro offers the ability to potentially improve embryo development by altering the composition of the culture medium in the vicinity of the embryo, or perhaps altering surface characteristics such as hydrophilicity, which may have mechanical and chemical advantages over static culture on inert surfaces, especially with emerging dynamic culture systems. Specialized surface coatings using this approach can potentially more closely mimic the ‘moist’ environment of the female reproductive tract.

Additionally, through chemical–physical interactions, macromolecules within the female reproductive tract are hypothesized to support embryo cellular homeostatic mechanisms, imparting responsiveness or plasticity to the embryo (Hunter, 1994; Pool and Martin, 1994; Pool, 2002). Thus, implementation of specialized surfaces for in vitro culture may render embryos more competent to compensate for encountered homeostatic imbalances, such as osmolality or pH. Conversely, surface modifications could also prove detrimental due to these same alterations.

As mentioned, the female reproductive tract is coated with various compounds, including components of the ECM. These ECM molecules present in the oviduct and uterus are diverse and complex, but are often categorized into two main groups: glycosaminoglycans and fibrous glycoproteins. Glycosaminoglycans are negatively charged polysaccharides chains of variable length, which, with the exception of hyaluronic acid, are covalently linked to a protein to form proteoglycans. Fibrous glycoproteins are often grouped into structural types, like collagen and elastin, and adhesive types, such as laminin and fibronectin. The majority of ECM compounds have been found to influence growth, function or differentiation in vitro in a variety of adherent somatic cell systems (Adams and Watt, 1993) and may influence preimplantation embryo development and function as well. Addition of fibrous glycoprotein components of the ECM, such as fibronectin and laminin, to culture media has varying effects on preimplantation embryo development. Laminin at varying doses was detrimental and decreased mouse blastocyst cell number, while fibronectin could support, but not improve mouse embryo development compared with control treatments (Figueiredo et al., 2002). Interestingly, fibronectin and laminin at 50 μg/ml improved human blastocyst hatching rates (Turpeenniemi-Hujanen et al., 1995), indicating perhaps species-specific actions, or importance of glycoprotein source and/or concentration. Additionally, glycosaminoglycans present in the female reproductive tract have also been added to culture media to explore impact on resulting embryo development. Heparin, hyaluronic acid and chondroitin sulfate at 0.5 mg/ml improved blastocyst development in IVF-derived bovine embryos over controls (Jang et al., 2003). Hyaluronan and heparin sulfate were found to be adequate substitutes for serum protein in supporting mouse blastocyst development, although chondroitin sulfate yielded fewer cell numbers (Figueiredo et al., 2002). Several other glycoproteins have been identified within the female reproductive tract of various species (Aviles et al., 2010) and these compounds, such as mucins and oviduct-specific oviductins, remain to be studied in regard to their impact on in vitro embryo development.

Importantly, simply dissolving or suspending these ECM or other compounds in culture media may not be the most appropriate method of utilizing these molecules. Macromolecules may be assuming a tertiary structure in solution that is not assumed when attached to a fixed site as in vivo. Thus, a compound with biological activity when bound to a surface may not convey this same effect when in solution and vice versa. Interestingly, many of the polyhydroxy compounds found in ECM have a polypeptide backbone with O-linked oligosaccharides projecting into an aqueous phase from fixed sites. When one considers that proteoglycans of the ECM, like heparin sulfate proteoglycan complexes, can act as anchors for a variety of growth factors and cytokines that impact embryo development including various insulin-like growth factors, fibroblast growth factors, transforming growth factor-β’s, vascular endothelial growth factor and others (Sakseln and Rifkin, 1990; Adams and Watt, 1993; Taipale and Keski-Oja, 1997), one can appreciate how proper orientation of these anchor compounds on a surface may be important for proper presentation of the substrates. Specific ECM compounds may influence development through regulating supply and activity of growth factors or cytokines and activity of signaling pathways within the embryo. If surface topography and contact with embryos, or modification of fluid microenvironments by compounds like glycosaminoglycans or proteoglycans, are causative factors for improvement of embryo development, then exploration of surface modifications used during in vitro embryo culture to mimic this in vivo environment may be beneficial. Limited work toward this end has been pursued.

**Matrigel**

Matrigel is a solubilized basement membrane preparation extracted from mouse sarcoma, a tumor rich in ECM proteins. Major components include laminin, collagen IV, heparin sulfate proteoglycans,
entactin and nidogen, as well as various growth factors, although these are not oriented in any particular fashion. Using an F1 hybrid mouse strain and HTF media, it was shown that culturing 15 embryos/50 μl on Matrigel diluted 1:8 (12.5%)-coated culture plates increased rates of mouse blastocyst hatching at 96 and 120 h from the 2-cell stage compared with embryos cultured in media alone, although total rates of blastocyst formation were similar (Carnegie et al., 1995). A later study culturing 10–30 embryos/600 μl found similar beneficial results using frozen/thawed 2-cell hybrid mouse embryos, with 0.8% Matrigel yielding higher rates of blastocyst development in one media (51.7 versus 32.1%) and increased hatching in another (67.9 versus 32.1%) (Lazzaroni et al., 1999). However, subsequent studies examining the ability of 1:8 diluted Matrigel to support zygote development from random-bred mouse strains, which experienced the 2-cell block, demonstrated an inhibitory effect of Matrigel on blastocyst development and hatching (Dawson et al., 1997). This deleterious effect was not observed with culture of 2-cell embryos. Thus, mouse strain and cell stage are important factors in efficacy of Matrigel, and it is unknown how other mammalian species react to the surface coating. Though Dawson and coworkers tested two different lots of Matrigel and noticed no difference, lot-to-lot variation may still be a factor in embryo toxicity.

Hyaluronic acid

Although conflicting data exist, inclusion of hyaluronic acid in culture media has been found to be beneficial for mouse and bovine embryo and fetal development (Gardner et al., 1999; Lane et al., 2003; Palasz et al., 2006), and improves pregnancy and implantation rates when included in human embryo transfer media (Korosec et al., 2007; Loutradi et al., 2007; Hambiliki et al., 2009; Sifer et al., 2009). At least one preliminary abstract has examined the effects of coating the culture surface with a glycosaminoglycan matrix of hyaluronic acid. Although coating of flat PDMS surfaces and microwells with hyaluronic acid hydrogels was able to support mouse blastocyst formation at comparable levels to uncoated flat PDMS surfaces (81.4 versus 72.6 versus 86.3%, respectively), it significantly decreased blastocyst cell numbers compared with no coating (Oakes et al., 2009). Furthermore, unpublished data from the author’s laboratory also indicated rotational culture in microwells coated with hyaluronic decreased blastocyst development compared with culture on a flat-coated surface. This study is one of the first attempts utilizing a specialized surface composed of proteoglycans in conjunction with a culture approach that maximizes surface area contact with the zona pellucida of developing embryos. However, it is obvious that other parameters need to be explored to elucidate benefits or detriments of this approach.

Agarose

Although not composed of ECM components, surface coatings using agarose have been used in supporting embryo development in vitro. Microwells made in agarose have been described for use in individually culture zona-free embryos (Peura and Vaja, 2003). This method does allow for easy access to and identification of embryos, although no immediate benefit on growth over traditional culture surfaces has been noted. It should be noted that agarose gel tunnels were also used for extended culture of post-hatching bovine embryos (Brandao et al., 2004). Interestingly, several types of agarose gel exist and it is unknown whether specific properties of these varying compounds can convey differential effects on embryo development.

Co-culture

Although widely used in the past for embryo culture, with the movement toward use of a defined media, co-culture has received less and less attention. Thought to be beneficial due primarily to modification of media chemical or ionic composition though secretion of various factors, co-culture also provides surface modifications and exposure to ECM and other compounds that may influence embryo development. For example, secreted glycoproteins from oviductal epithelium culture have been found to associate with the zona pellucida, as well as incorporate in the perivitelline space and blastomere surfaces in several species (Gandolfi et al., 1989, 1991; Boice et al., 1990; Buhi et al., 1993), although it is unclear if direct contact was influential for this interaction. However, subsequent studies attempting to supplement media with these type of oviductal secretions to improve embryo development have met with mixed success (reviewed by Bavister, 1988; Aviles et al., 2010), possibly due to reasons described above. Interestingly, co-culture of human embryos on autologous cumulus cells resulted in accumulation of cells around the embryo, engulfing it on all sides (Parisik et al., 2006). Additionally, formation of ‘dendritic processes’ extending toward cleaving embryos was also observed, which may alter the physical environment and affect development. However, studies using mouse embryos and Vero cell co-culture demonstrated that no differences were apparent in rates of blastocyst formation when embryos were cultured directly on the cells, or cultured directly above the cells, separated by a porous membrane (Lee et al., 2001), indicating direct contact with surface molecules may not be required or beneficial. Most recently, autologous endometrial cell co-culture has yielded promising results for improved human embryo development in vitro (Jayot et al., 1995; Barmat et al., 1999; Eyheremendy et al., 2010), although any benefit of surface contact is unknown. Thus, any definitive specific physical role of co-culture remains unclear, but is likely to be dependent upon co-culture cell type or species of embryos.

Three-dimensional matrix

Certain limitations exist with two-dimensional culture, as contact with any specialized surface is minimal. Through the use of three-dimensional culture approaches, not only are cells provided more structural support, but also offered more direct physical interaction with their surrounding environment, similar to that experienced in vivo. Additionally, three-dimensional culture may more appropriately allow for embedding and orientation of an array of glycoproteins or other macromolecules, compared with culture on a two-dimensional surface (Fig. 5). To date, most of the work with three-dimensional culture has been performed with follicles or oocytes, but similar approaches could be used with embryos, although modifications of the approach may be required to allow embryo visualization and grading, as well as embryo recovery for embryo transfer. Regardless of these current limitations, compounds currently used in three-dimensional culture such as collagen (Combelles et al., 2005; Vanhoutte et al., 2009a, b), alginate (Pangas et al., 2003; Kreeger et al., 2006; Torre et al., 2006; Xu et al., 2006, 2009; West et al.,
Specialized surface coatings offer an intriguing means of potentially improving current in vitro embryo culture. However, use of biologic materials does require consideration of purity and potential contaminants. In this respect, suitable synthetic compounds may be more attractive. Currently, no commercially available specialized embryo culture platform with such coatings exists. Furthermore, there is a lack of information regarding surface coatings and impact on human embryo development. Therefore, it is difficult to draw any conclusions regarding their benefit or true potential and future work is required. One consideration is that this approach may have more of an impact when used with culture platforms that increase surface area contact points. Other issues deserving attention entail physical presentation of macromolecules to gametes, as this is currently problematic. Pool (2002) stated, ‘to mimic the physical–chemical conditions present in vivo, a potential culture system must be able to present an array of macromolecules, an assemblage that changes qualitatively, with time, from fixed sites in a minimum volume of water’. Therefore, it is likely not simply the surface material the embryos are cultured on that has an impact, but rather the composition, or more specifically, the orientation of included/embedded compounds that may convey the most benefit (Fig. 5). However, an important consideration is that the zona pellucida barrier could hinder interactions of preimplantation embryos with these specialized surfaces or embedded elements. Although a few surface materials have been examined for their ability to support embryo development, more detailed experiments examining novel surface coatings, such as various hydrogels with varying polarity or polyhydroxylated compounds such as DHEA-dextran, as well as various compliments and configurations of embedded macromolecules may prove insightful. Several proteoglycans and oviductal-specific glycoproteins, like oviductin, have been identified (see review Aviles et al., 2010), and there are a multitude of polymeric biomaterials that remain to be studied as well (Baldwin and Kiick, 2010; Krishna and Kiick, 2010) with respect to their role in preimplantation embryo development in vitro.

Figure 5 Future directions for improvement of embryo culture platform. Use of specialized surface coatings in combination with approaches that maximize cell-surface contact (microchannel, microfunnel or microwell) may allow for embedding and orientation of macromolecules that could convey benefit to embryo development in vitro.

Summary
Specialized surface coatings offer an intriguing means of potentially improving current in vitro embryo culture. However, use of biologic materials does require consideration of purity and potential contaminants. In this respect, suitable synthetic compounds may be more attractive. Currently, no commercially available specialized embryo culture platform with such coatings exists. Furthermore, there is a lack of information regarding surface coatings and impact on human embryo development. Therefore, it is difficult to draw any conclusions regarding their benefit or true potential and future work is required. One consideration is that this approach may have more of an impact when used with culture platforms that increase surface area contact points. Other issues deserving attention entail physical presentation of macromolecules to gametes, as this is currently problematic. Pool (2002) stated, ‘to mimic the physical–chemical conditions present in vivo, a potential culture system must be able to present an array of macromolecules, an assemblage that changes qualitatively, with time, from fixed sites in a minimum volume of water’. Therefore, it is likely not simply the surface material the embryos are cultured on that has an impact, but rather the composition, or more specifically, the orientation of included/embedded compounds that may convey the most benefit (Fig. 5). However, an important consideration is that the zona pellucida barrier could hinder interactions of preimplantation embryos with these specialized surfaces or embedded elements. Although a few surface materials have been examined for their ability to support embryo development, more detailed experiments examining novel surface coatings, such as various hydrogels with varying polarity or polyhydroxylated compounds such as DHEA-dextran, as well as various compliments and configurations of embedded macromolecules may prove insightful. Several proteoglycans and oviductal-specific glycoproteins, like oviductin, have been identified (see review Aviles et al., 2010), and there are a multitude of polymeric biomaterials that remain to be studied as well (Baldwin and Kiick, 2010; Krishna and Kiick, 2010) with respect to their role in preimplantation embryo development in vitro.

Conclusion
The physical environment during in vitro embryo culture can affect resulting embryo quality. Examination of these physical requirements through development of novel culture platforms may lead to new approaches to future help improve ART. It is likely that these new platforms will call for deviation from current protocols utilizing small Petri dishes or test tubes placed into large gas-filled incubators. Rather, they may evolve to mimic the environment encountered in vivo. These emerging platforms and approaches can take advantage of improvements in engineering and conceivably utilize stand-alone culture devices, without the need for large external incubators, which take advantage of specific physical properties and may even produce their own atmosphere. Indeed, embryos can be cultured with atmosphere created via chemical reactions (Suzuki et al., 1999; Avery et al., 2000; Swain, 2010). One can envision combination of a perfusion system, such as a microfluidic device, with emerging three-dimensional culture or specialized surface coatings, housed in a way as to generate its own atmosphere and heat. Furthermore, the true power of these platforms is the potential to incorporate real-time analysis through use of nanosensors or other probes. As non-invasive diagnostics evolve to aid us in better determining embryo quality, integration of these assays into an improved culture platform will prove invaluable to improvement of embryo culture and resulting of IVF success rates.

Authors’ roles
Both J.E.S. and G.D.S. contributed to the conception and design as well as the writing and revising of this review article. Furthermore, both authors have approved the final version.

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