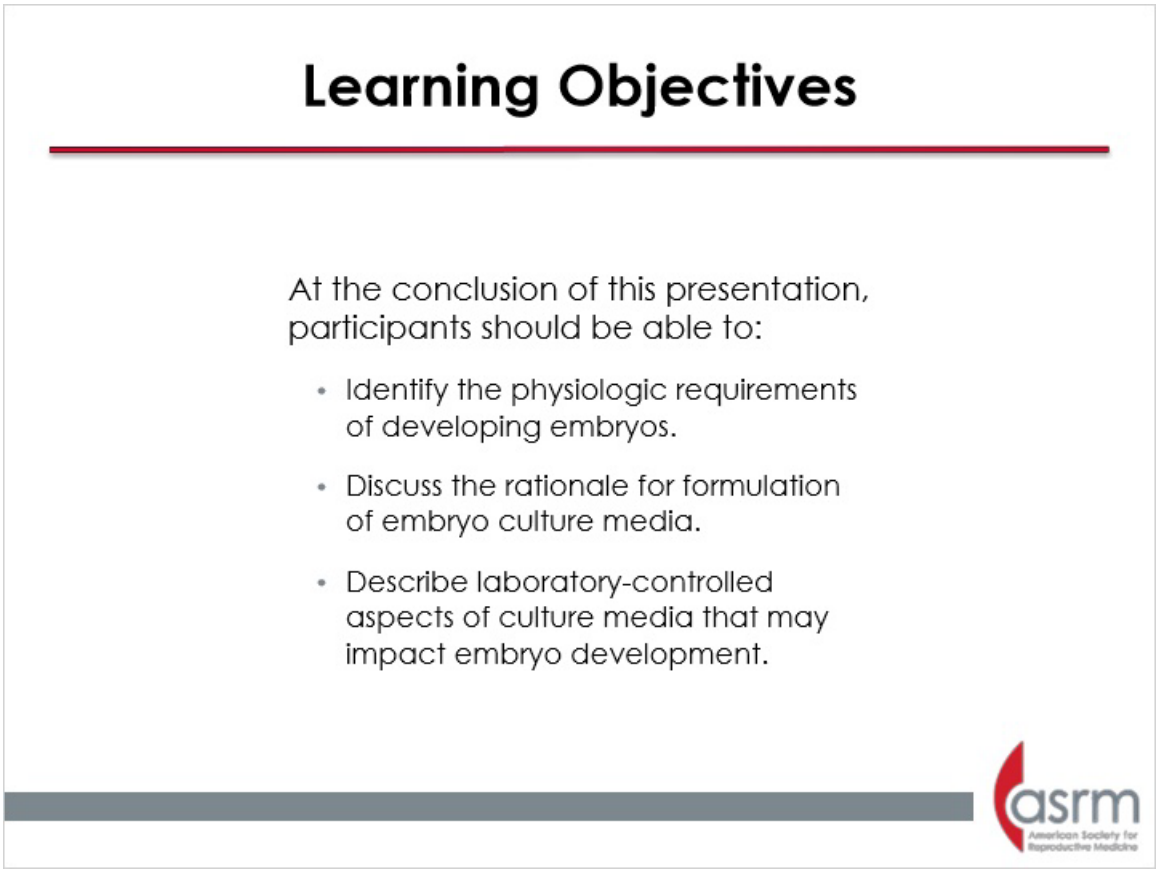
LABCC100 Lesson 12

1.1 Culture Media and Conditions, Part 1

Notes:

Welcome to the American Society for Reproductive Medicine's eLearning modules. The subject of this presentation is Culture Media and Conditions, Part 1.

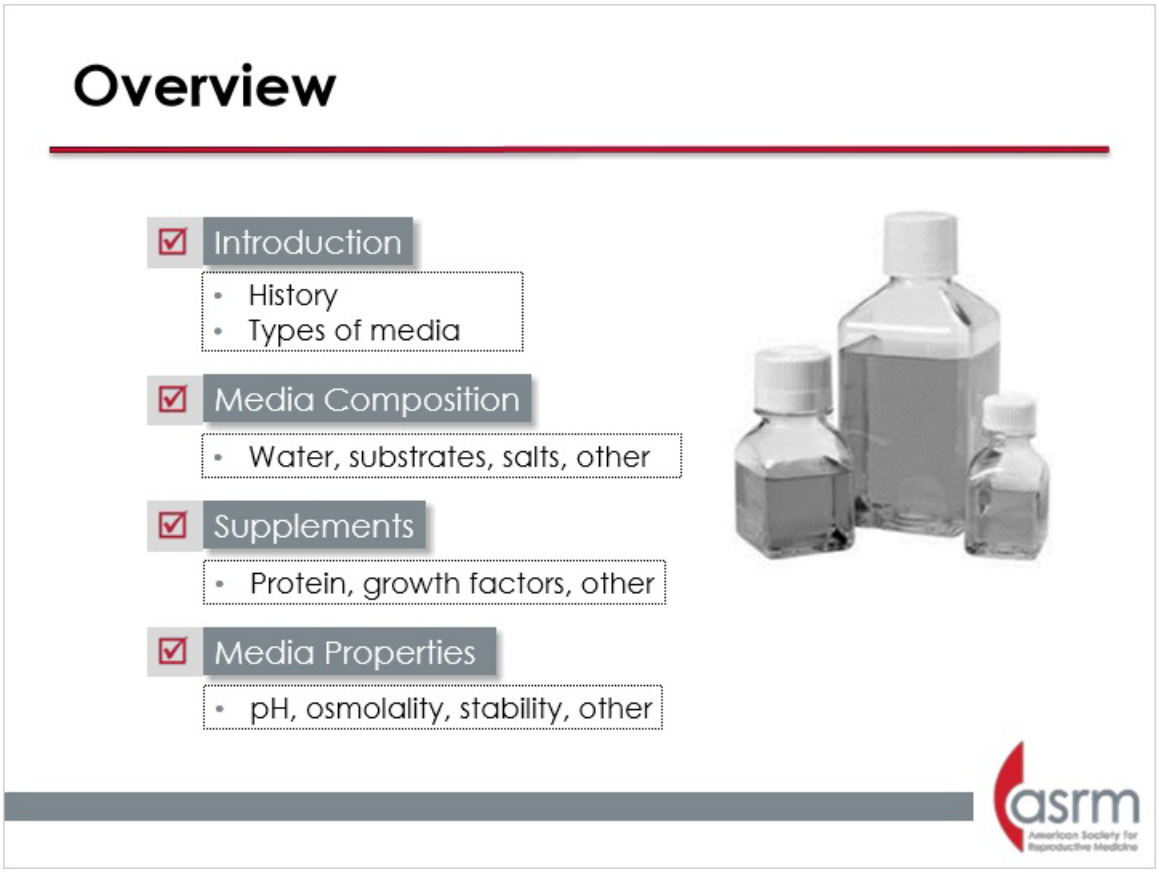
1.2 Learning Objectives



Notes:

At the conclusion of this presentation, participants should be able to:
Identify the physiologic requirements of developing embryos.
Discuss the rationale for formulation of embryo culture media.
Describe laboratory-controlled aspects of culture media that may impact embryo development.

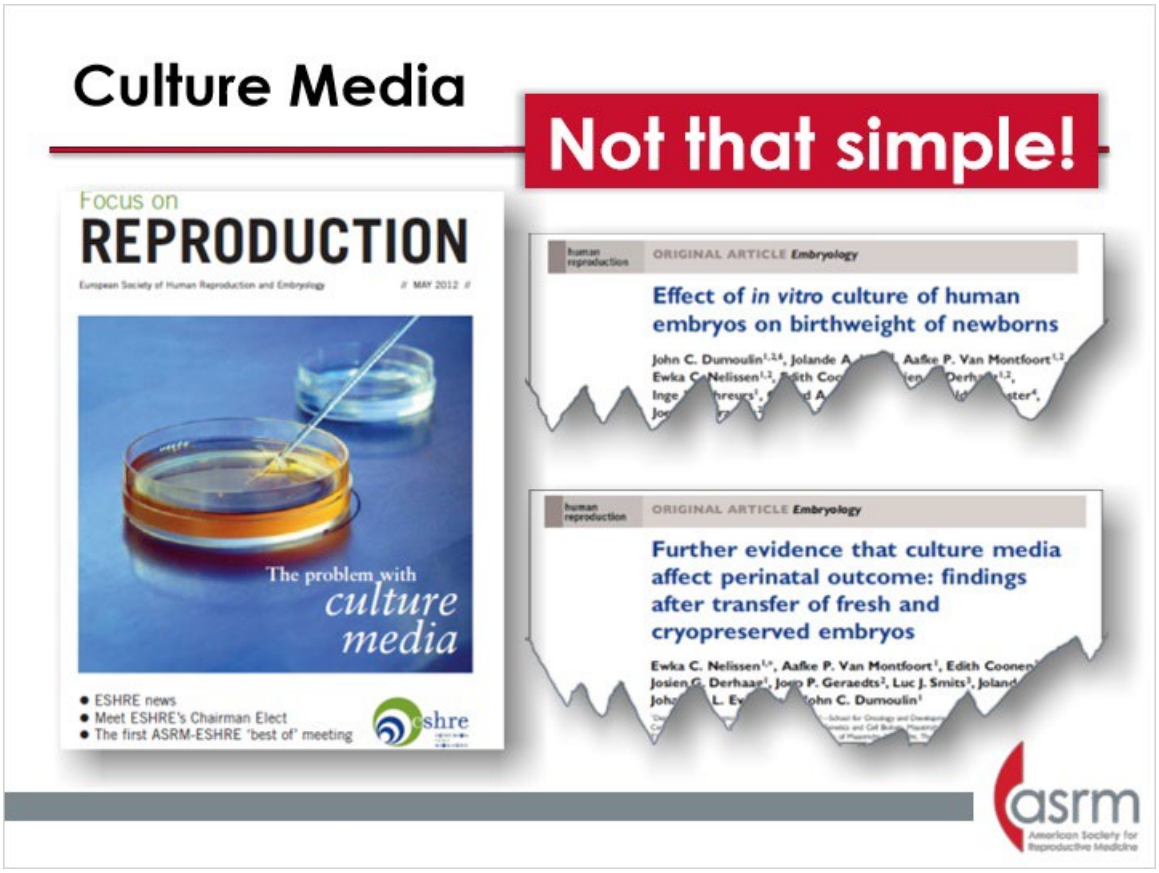
1.3 Overview



Notes:

This presentation will begin with an introduction to culture media, its history, and the various types that have been developed for use in assisted reproduction. Also covered will be the composition of media, including water, substrates, salts, and other substances, as well as media supplements such as protein and growth factors. Also, physical and chemical properties of media, including pH, osmolality, and stability will be addressed.

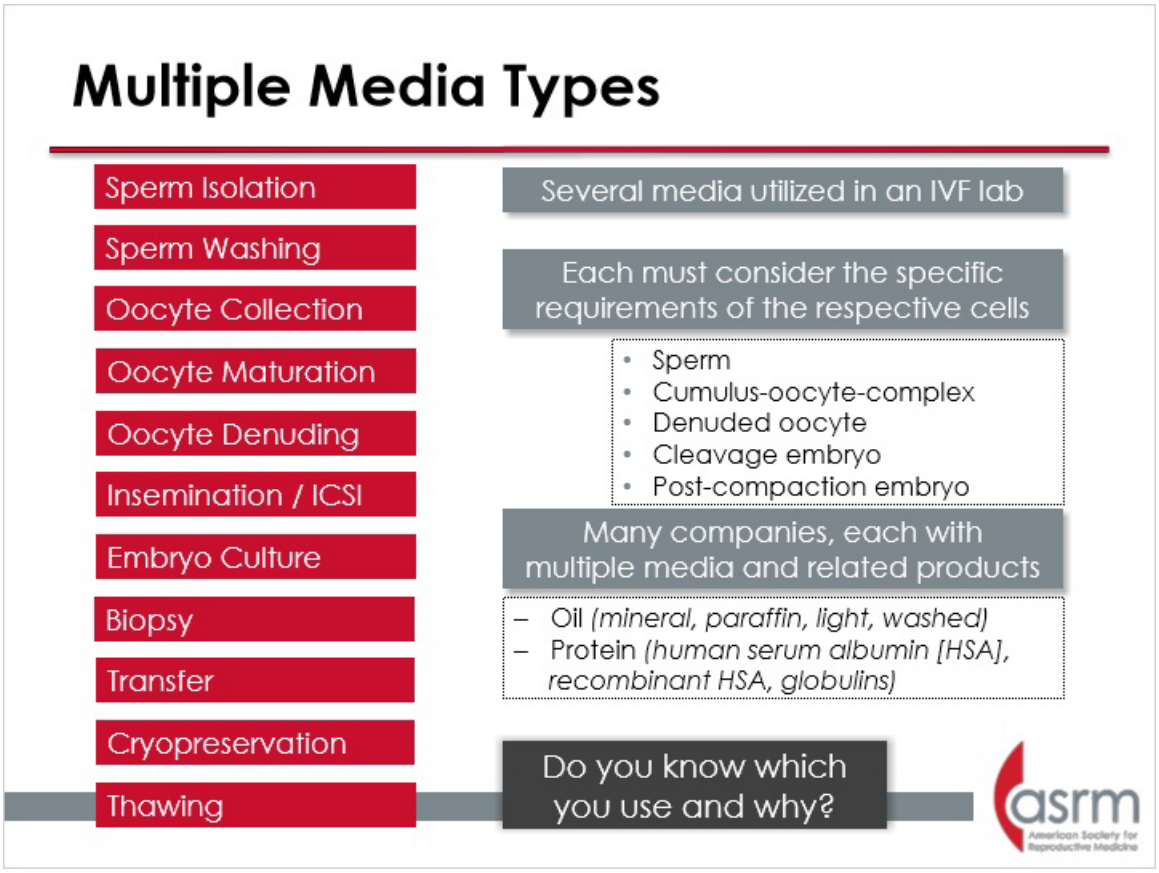
1.4 Culture Media



Notes:

The use of culture media in the in vitro fertilization (IVF) lab has long been an area of extreme interest, with numerous studies attempting to optimize the composition to improve embryo development and assisted reproductive outcomes. More recent studies that suggest an impact of embryo culture media on epigenetic status and the resulting health of offspring have renewed this interest and further reinforced the importance of this aspect of the culture system. Several studies, such as those shown here, have highlighted this effect. While there are some limitations in these particular studies, and they need to be interpreted with caution, the efficacy of embryo culture media is certainly an area that still deserves attention. It should be pointed out that placing blame on an inefficient culture system is not as easy as blaming the "culture medium."

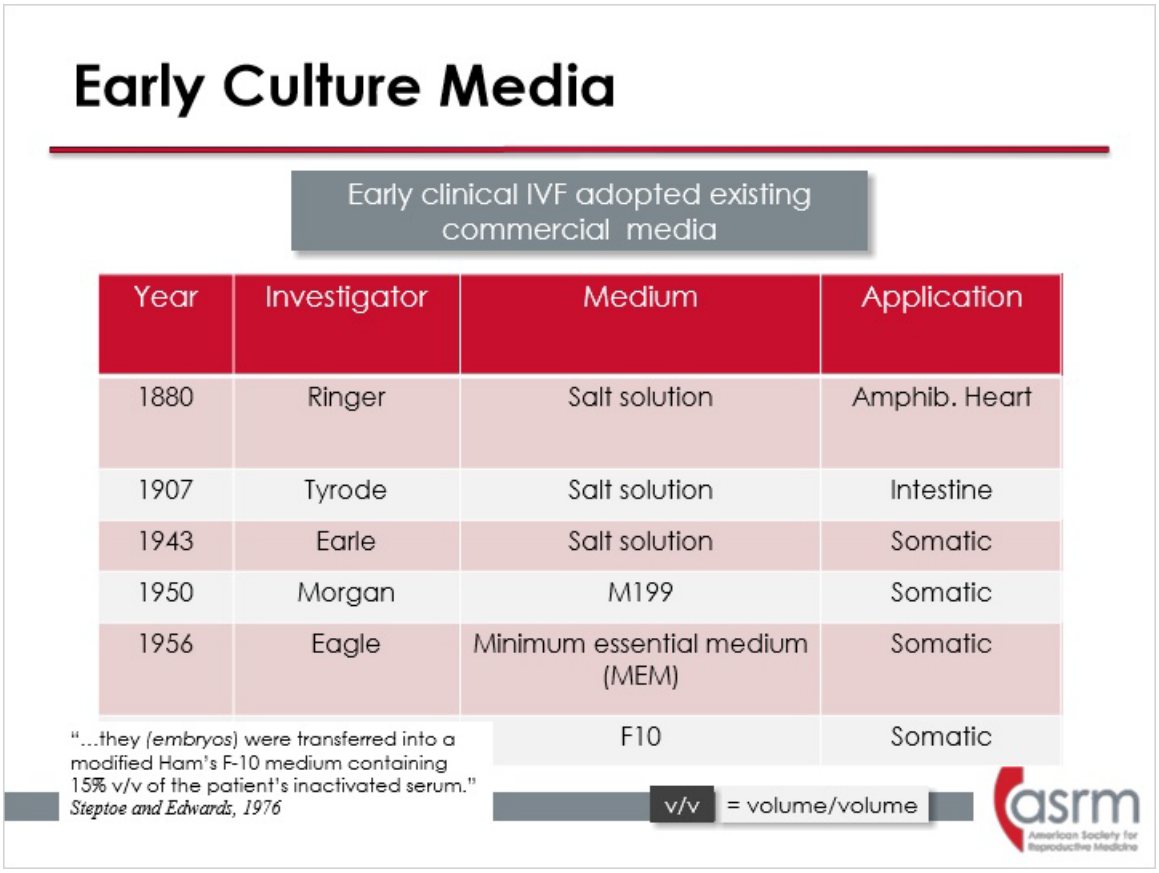
1.5 Multiple Media Types



Notes:

There are actually several media used within the IVF laboratory; these vary depending on the cell type or the particular procedural step. Furthermore, there are several companies that sell commercial culture media that vary, and there are numerous media "accessories" or supplements that accompany their use. For example, there are at least 4 types of mineral oil and 3 types of protein currently marketed. Each of these could have an impact on the embryo and resulting offspring. Considering all the variables involved with culture media as well as the numerous other variables within the culture system as a whole, one can begin to appreciate the oversimplification of trying to assign problems with IVF to a single culture medium. Thus, it is imperative to know how and why these media were formulated to begin to try to determine the impact of culture media.

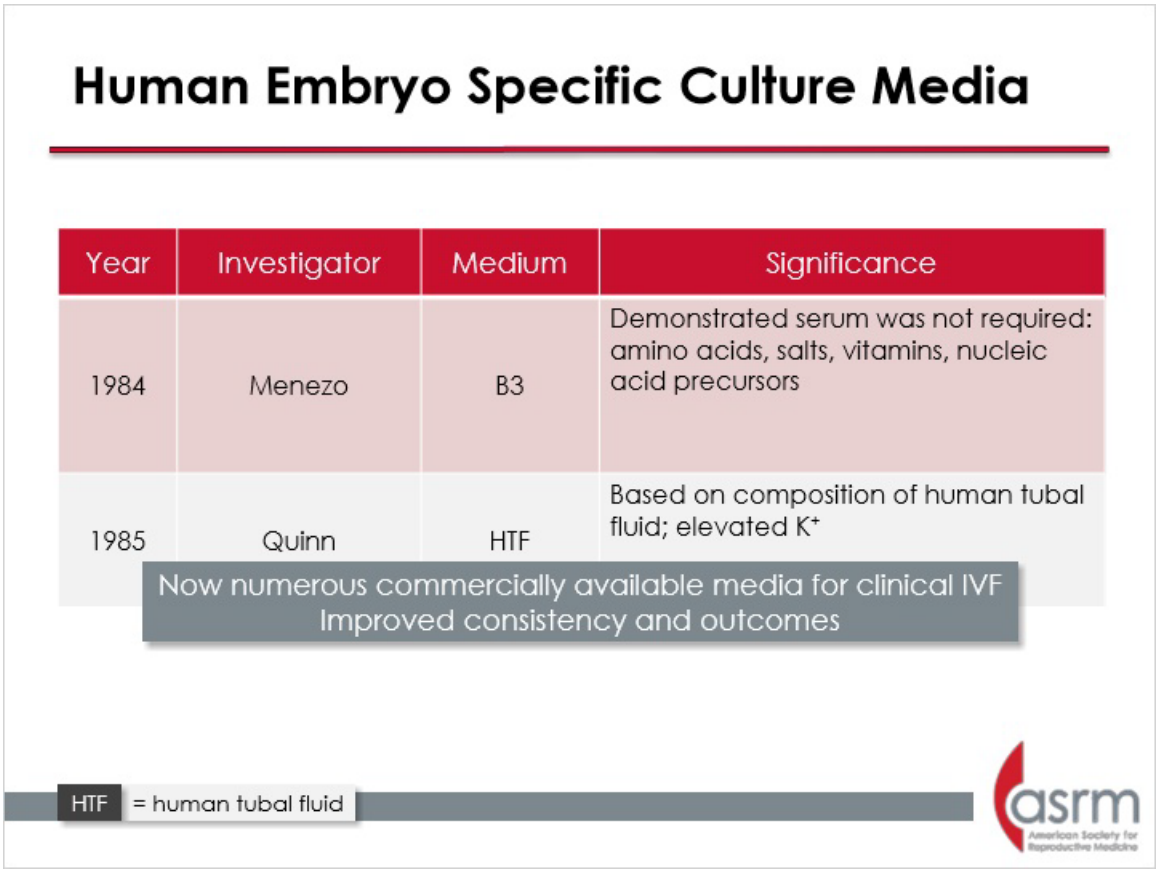
1.6 Early Culture Media



Notes:

Early attempts at growing human embryos in vitro relied on use of commercially available media that were largely developed for somatic cells. This table lists common culture media and their original application. At one point in time, each of these, and slight variations, were used for embryo culture. Indeed, as seen from the quote here, one of the first attempts by Steptoe and Edwards to grow human embryos in vitro utilized Ham's F-10; this report from the *Lancet* resulted in a tubal pregnancy. However, because these media were originally developed for other cell types, they were not overly efficient and changes were required for improvement.

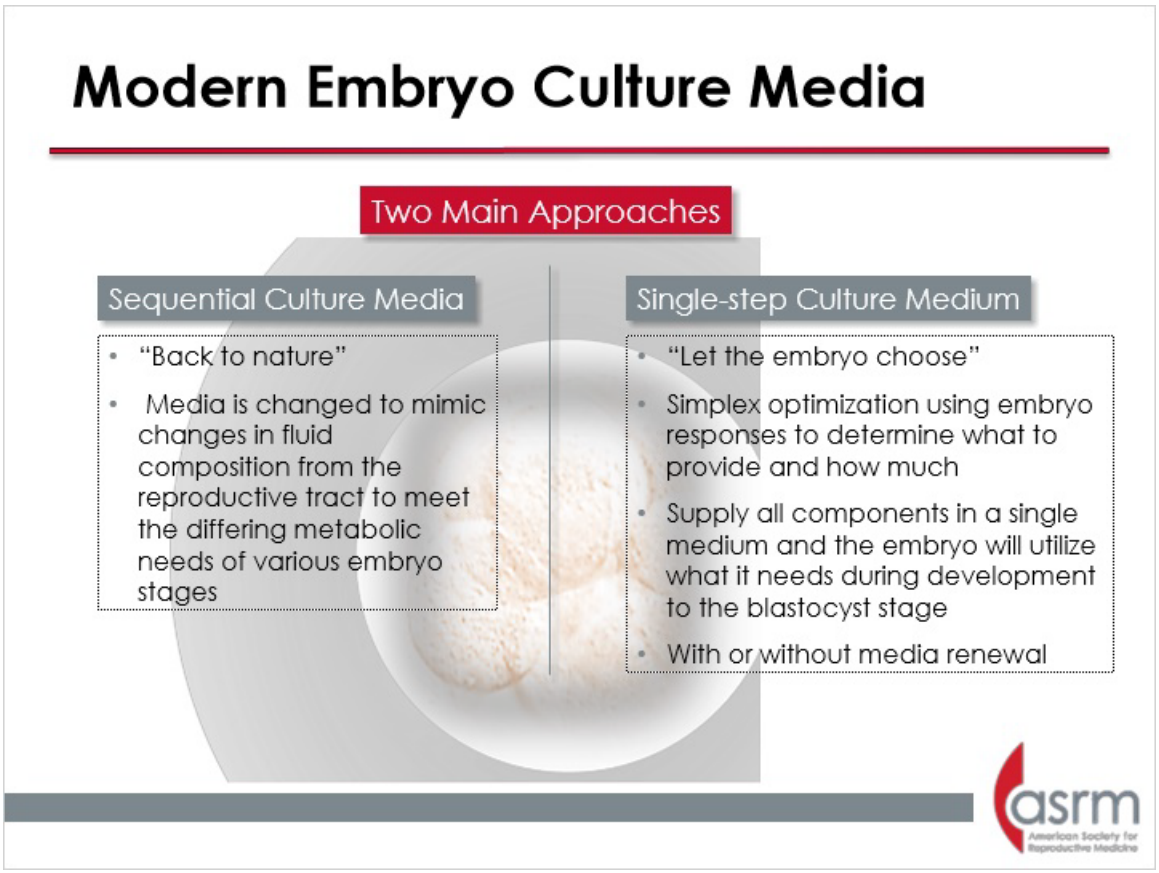
1.7 Human Embryo Specific Culture Media



Notes:

This need for improved culture medium efficacy resulted in media formulated specifically for the human embryo. There are numerous studies that could be cited that aided in the continued development and improvement of embryo culture media, including a library of work using mouse embryos…and certainly names like Whitten, Whittingham, and Brinster, deserve to be mentioned in this discussion, as well as Biggers and Bavister and other members of the so-called "Culture Club." After all, the rodent/mouse has served as one of the primary models for development of human embryo culture media, and these pioneers were instrumental in elucidating key components of embryo physiology and media formulations to overcome in vitro blocks to development. However, perhaps the two most significant papers in terms of media formulated specifically for human use include those of Menezo and Quinn. Menezo's 1984 paper on B3 media showed that embryos could be grown in vitro and pregnancies obtained without the use of serum, which was a large advancement at the time. Quinn's medium was based on the composition of human tubal fluid, hence the name HTF. This then led to formulation of numerous other media designed for clinical IVF and really has led to the paradigm enjoyed today, where media can be purchased commercially, which has dramatically improved consistency and outcomes.

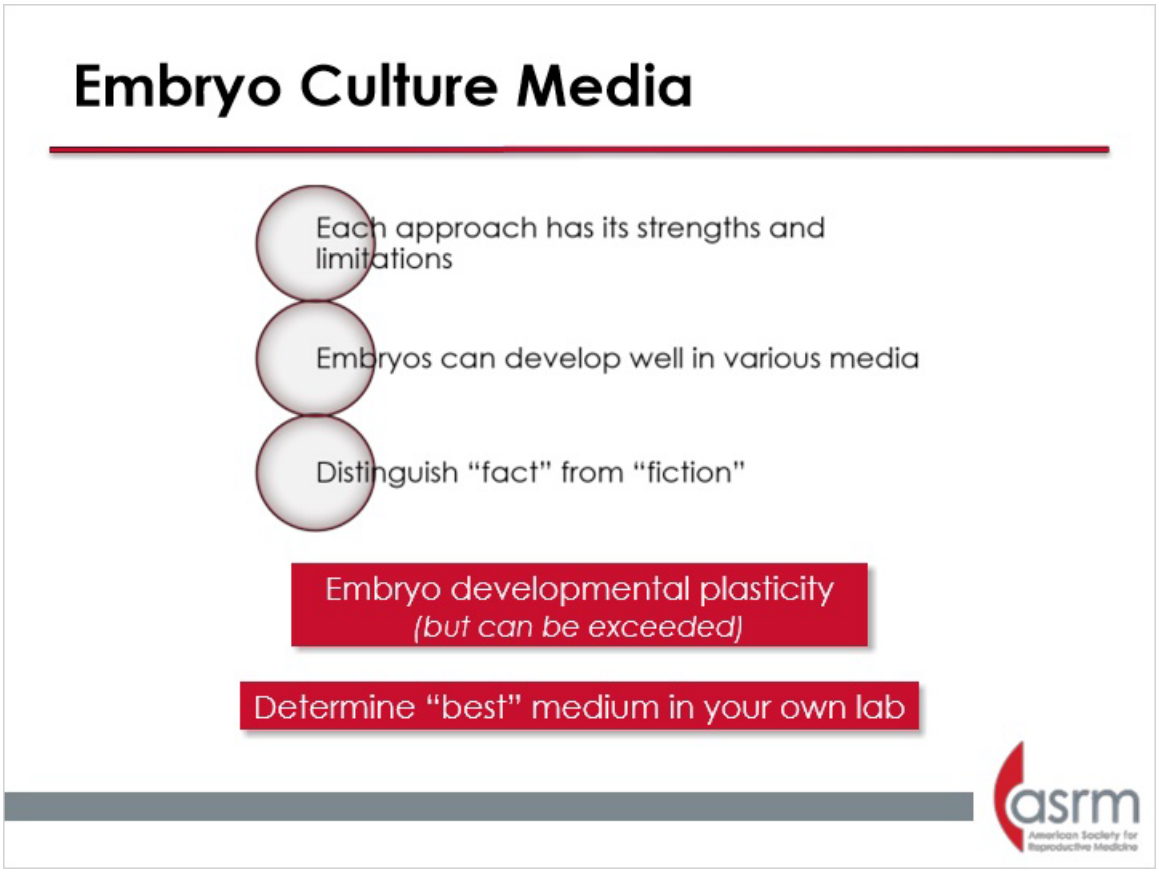
1.8 Modern Embryo Culture Media



Notes:

Modern embryo culture media are commonly divided into two main categories: sequential and single-step. Sequential media are often referred to as following the "back to nature" approach, where the media and composition are changed to mimic the proposed changes in fluid composition in vivo between the oviduct and uterus and to meet the changing metabolic needs of the developing embryo. This is perhaps best known to be associated with the formulation of G1/G2 media and the work of David Gardner and Michelle Lane. In contrast, single-step media follow the principle of "let the embryo choose," where all ingredients are supplied to the embryo in a single medium. This single-step approach can be utilized with uninterrupted culture over the 5-6 days in the lab, or with medium renewal around day 3 of development. This approach is really based on the work of John Biggers and the potassium simplex-optimized media with amino acids (KSOMaa).

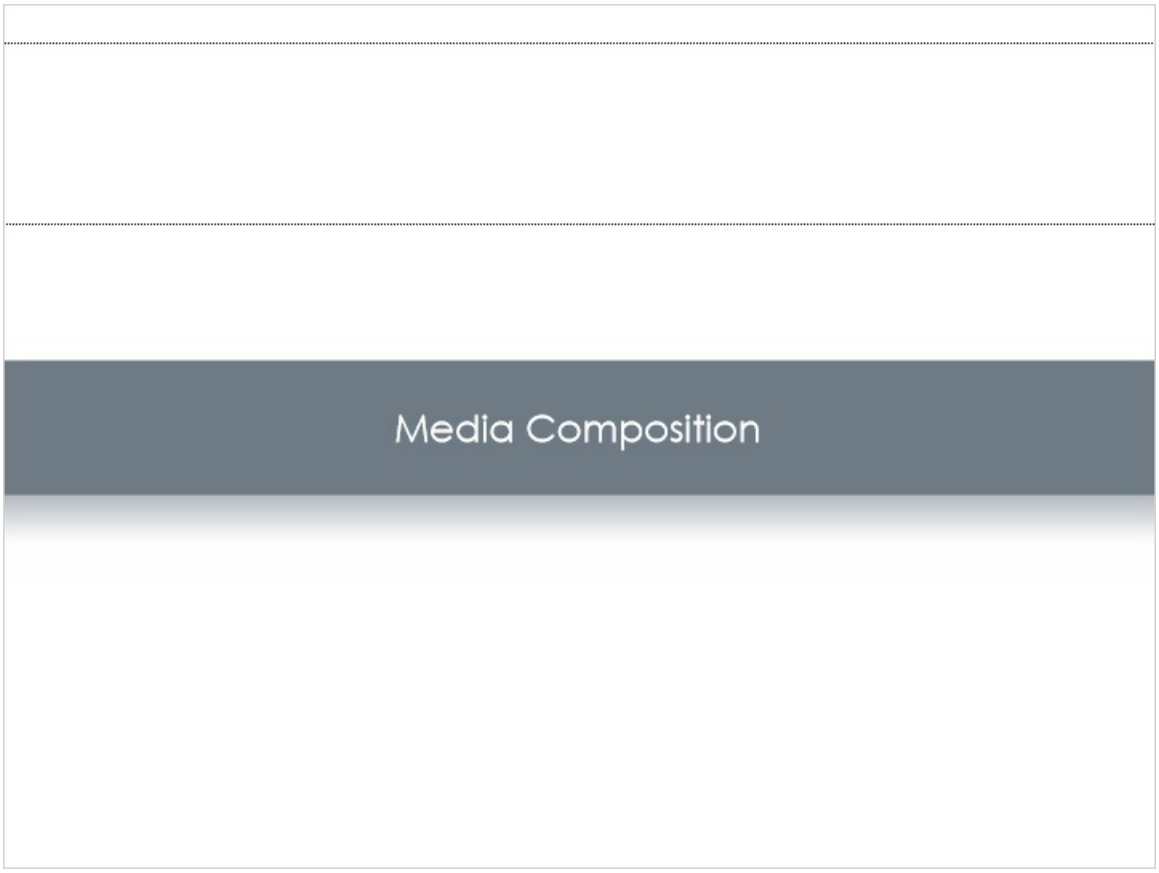
1.9 Embryo Culture Media



Notes:

It is important to note that each culture medium or approach has its own strengths and limitations. An in-depth coverage of this debate is beyond the scope of this presentation. However, it should be noted that embryos can develop equally well in various culture media. Also, it is important to understand the basis for media formulation to help distinguish fact from fiction when it comes to culture media efficacy. The reality is, embryos do have developmental plasticity and can adapt to their environment-though this can be exceeded-and the goal is to try to reduce the amount of stress to which the embryo is exposed. In reality, it is up to each lab to determine which product will perform best in the context of their entire culture system. As will be discussed, there are more considerations in the growth of high-quality embryos than just selection of the medium.

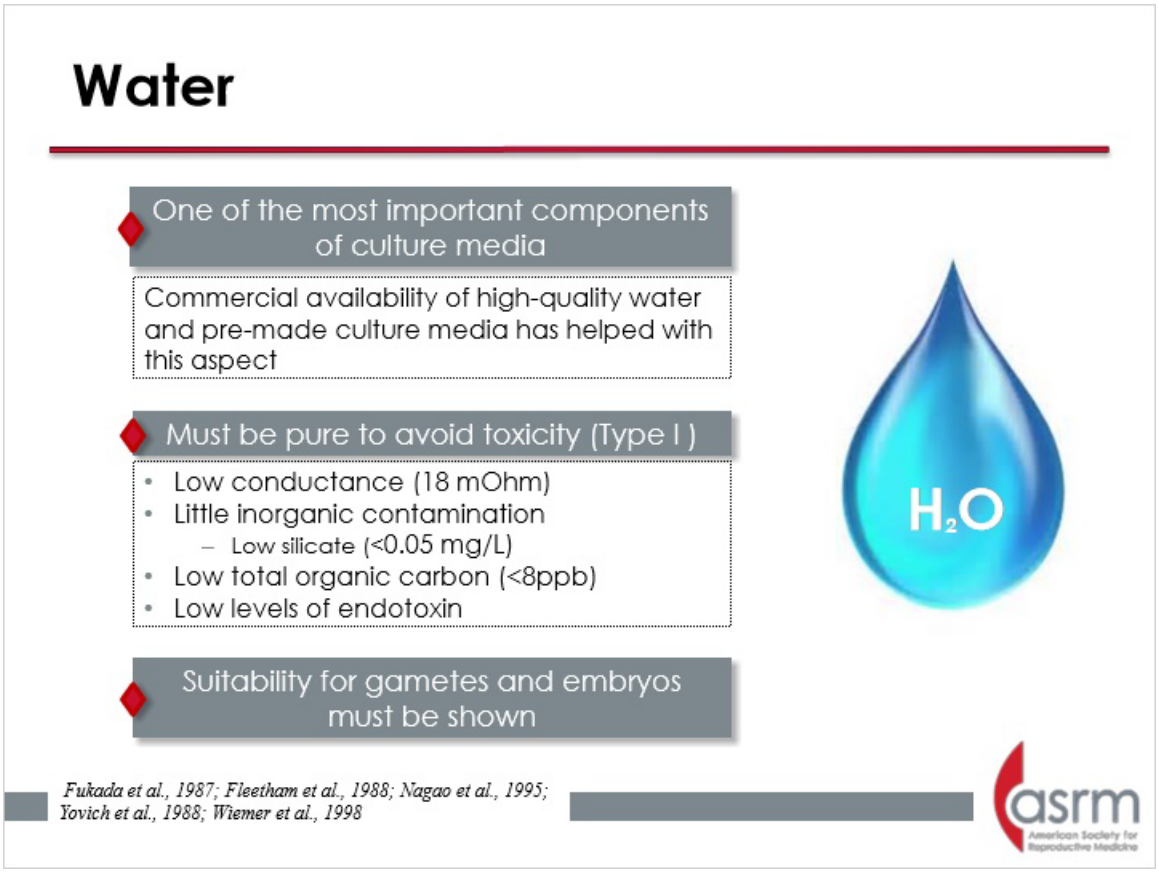
1.10 Media Composition



Notes:

This section will discuss media composition.

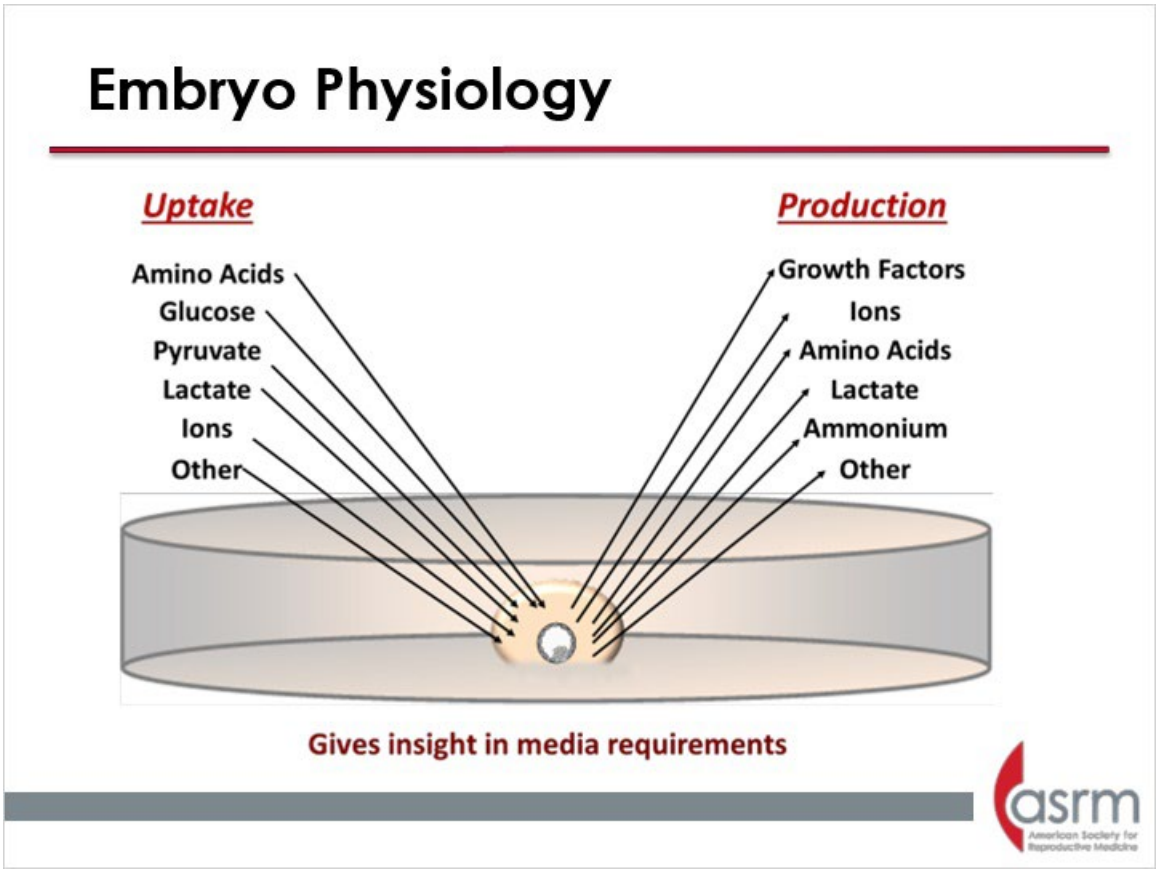
1.11 Water



Notes:

One of the most important considerations for culture medium formulation is use of high-quality water. This aspect tends to be taken for granted now due to the readily available nature of commercial media. However, water quality is imperative. Type 1 water is recommended. This is commonly referred to as “milliQ” water, although this is actually a commercial name. The resulting purified water is deionized and passed through a series of filters, including a 0.20µm filter. The final product should have low conductance and little inorganic ion contamination. It should also be free of organic compounds or high amounts of endotoxin. The water suitability should be verified using a sensitive bioassay. Various studies have examined the impact of various water types and have shown variability. Various reviews have discussed filtration methods and achievable levels of purity.

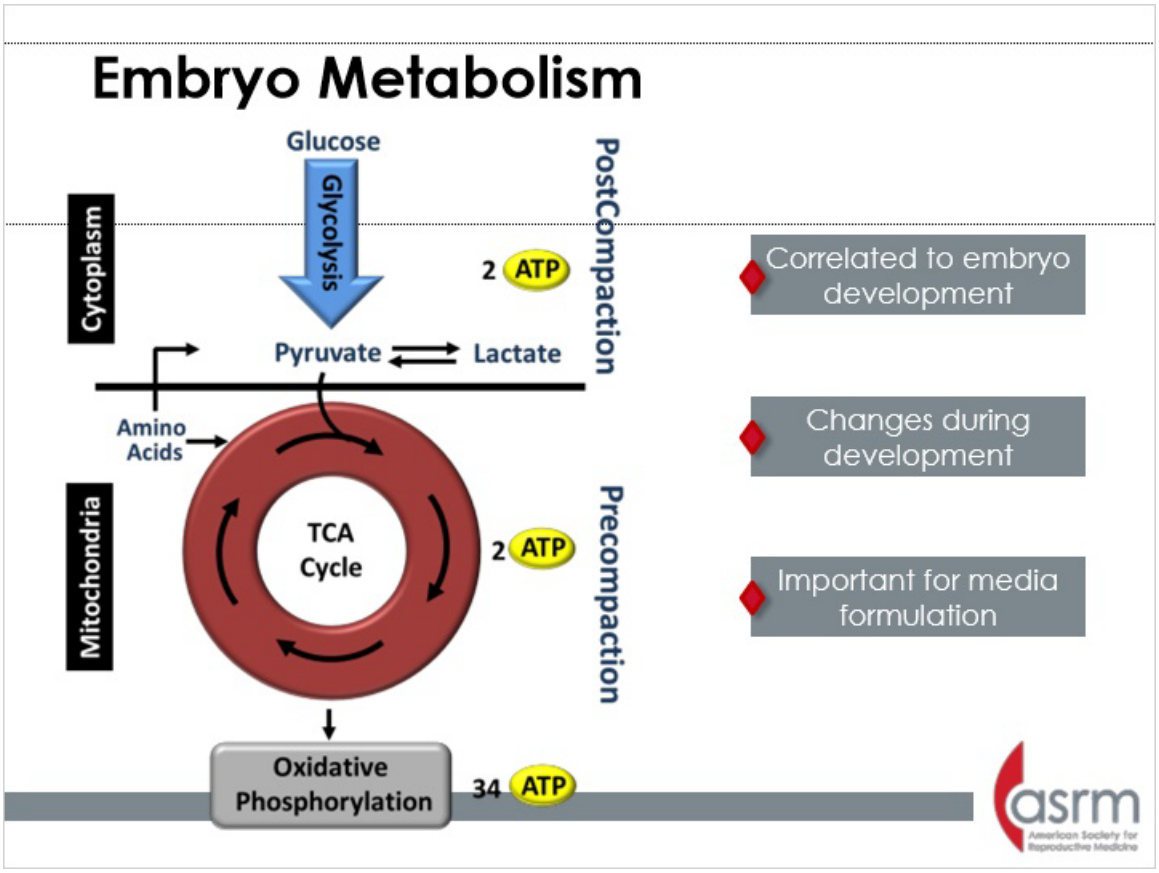
1.12 Embryo Physiology



Notes:

The formulation of embryo culture media must promote optimal embryo physiology. Studies of spent embryo culture media have shown that embryos take up and produce various factors into the culture medium. Using techniques such as ultramicrofluorescent assays and radiolabeled substrates, uptake and utilization, as well as production of substrates, have been assessed. This insight has played a critical role into media formulation.

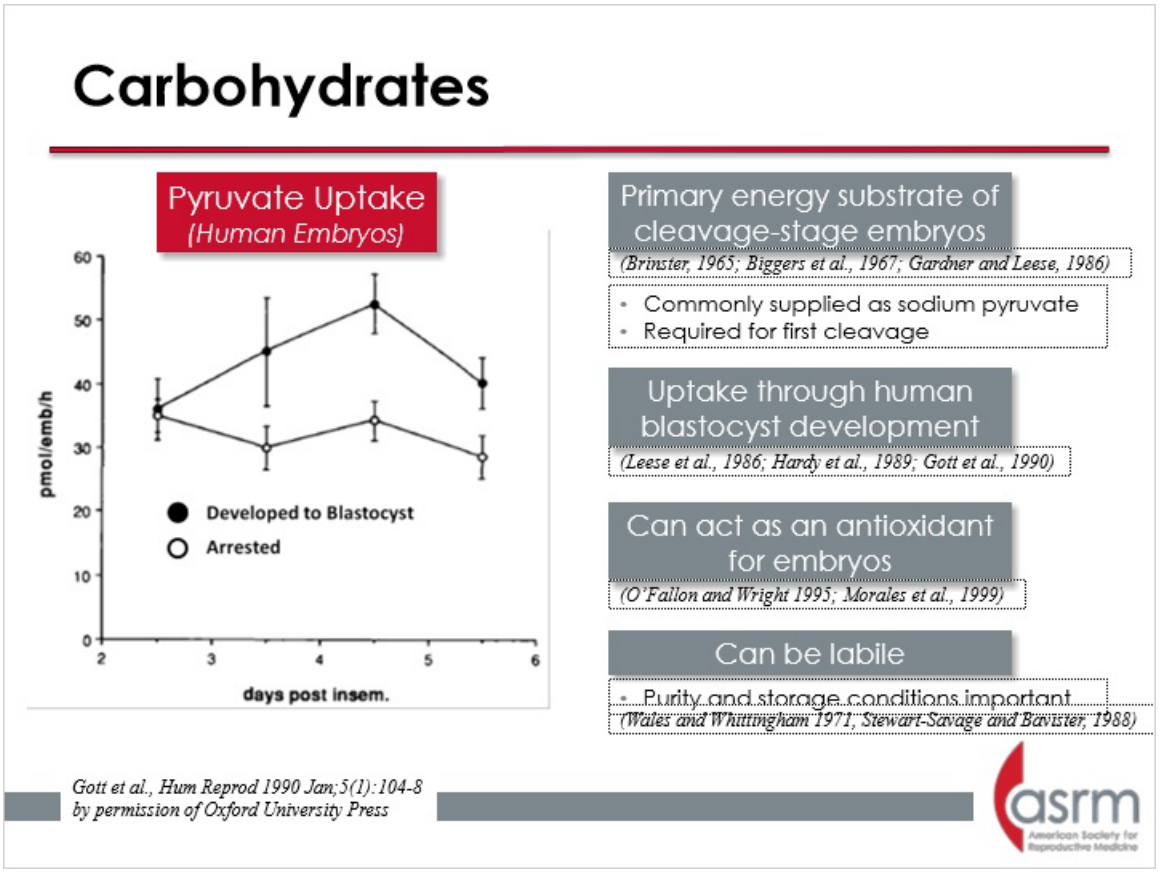
1.13 Embryo Metabolism



Notes:

It is well known that embryo metabolism correlates with embryo quality and developmental competence. Importantly, metabolism changes during development. Precompaction embryos primarily utilize substrates of the tricarboxylic acid or Kreb's cycle, while postcompaction stages of the embryo rely more heavily on glucose metabolism.

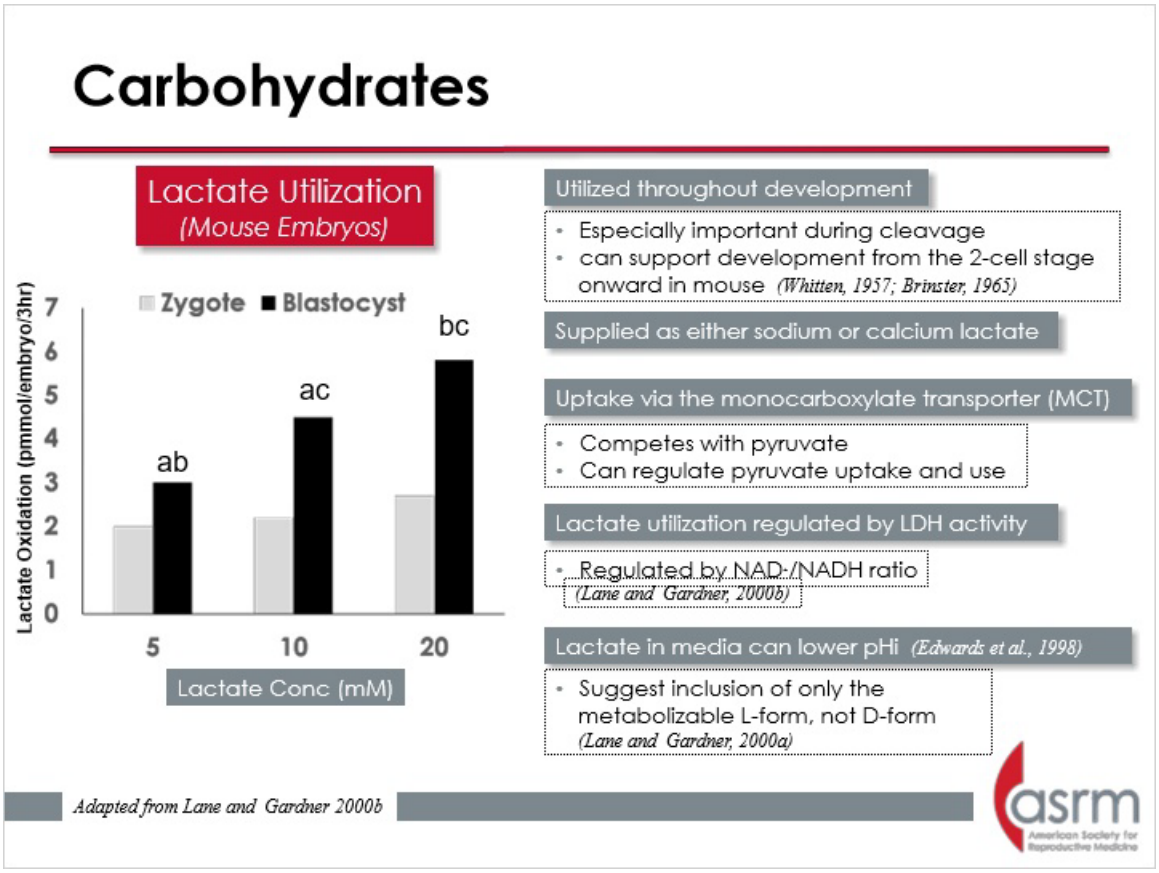
1.14 Carbohydrates



Notes:

How does this relate to formulation of modern embryo culture media? First, understand that carbohydrates are the main energy sources of embryos. Specifically, pyruvate is the primary substrate of cleavage-stage embryos. In fact, in the rodent, pyruvate was shown to be required for the first cleavage division. This being said, pyruvate is actually used throughout development and is an extremely important component of culture media. In addition to its role as an energy substrate, pyruvate may also play a role as an antioxidant. However, pyruvate can be labile and the byproducts of breakdown can impair metabolism and antioxidant properties. Thus, the pyruvate source and media storage conditions are important. It has been suggested that elevated pH could perhaps hasten pyruvate breakdown, which has lead some labs to limit the time that media may be used once opened.

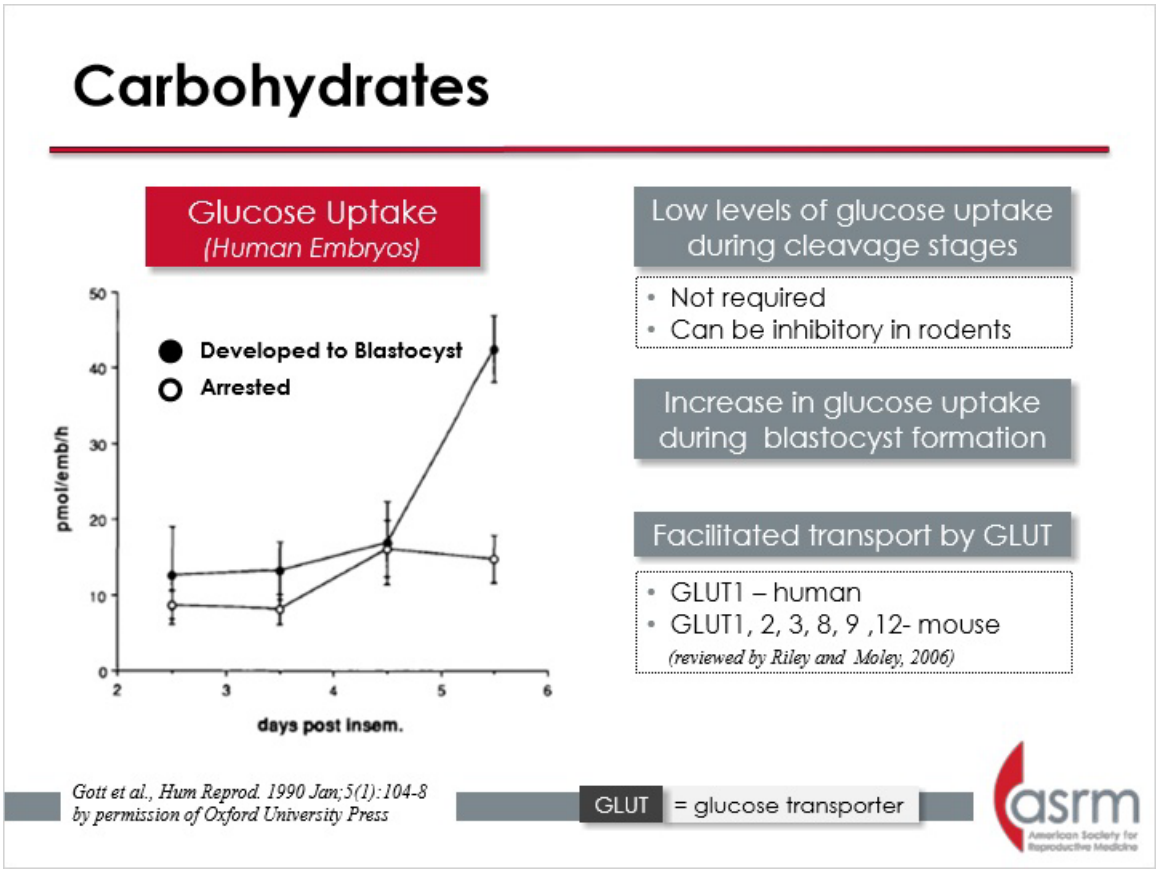
1.15 Carbohydrates



Notes:

Lactate is also an important substrate in embryo culture media. Like pyruvate, lactate is used throughout development, but is especially important for cleavage-stage embryo development. Lactate can support blastocyst formation after the 2-cell stage. It is taken up via the monocarboxylate transporter and therefore competes with pyruvate uptake. In fact, the concentrations of lactate in the media can impact pyruvate metabolism. This illustrates how complex embryo metabolism is and how difficult it can be to alter media composition to gain the desired beneficial effect. Lactate use is regulated by lactate dehydrogenase (LDH). In addition to its metabolic role, lactate in media lowers the internal pH of the cell (pHi). As will be shown later, pHi is an important factor to consider. For this reason, it is suggested that only the metabolizable L-form of lactate be used in media.

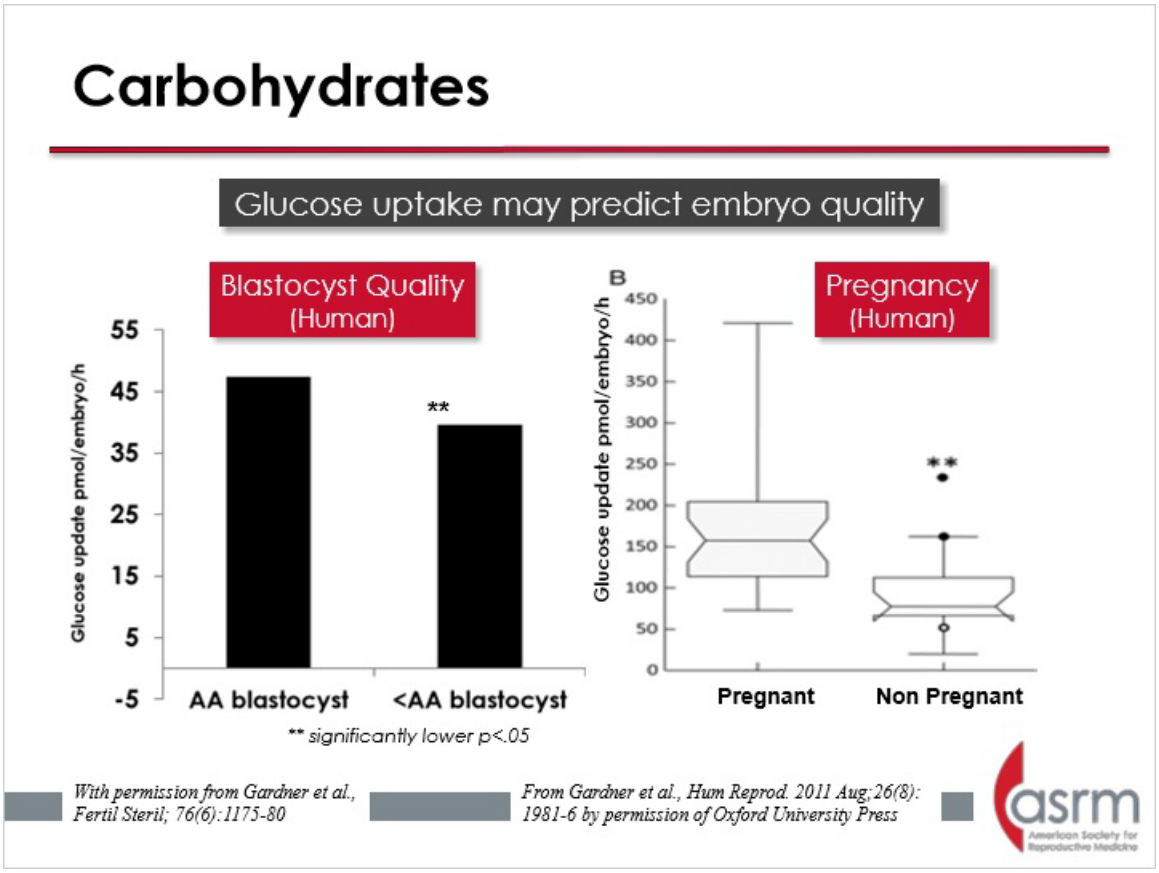
1.16 Carbohydrates



Notes:

Perhaps the most discussed energy substrate in embryo culture media is glucose. During early cleavage, glucose is taken up at low levels, although it is not required for development and can be detrimental at elevated concentrations in some species. Glucose uptake increases markedly around the time of compaction to meet the increased energy demands of compaction and blastocyst formation. Glucose uptake is via facilitated transport by membrane-bound GLUT transporters.

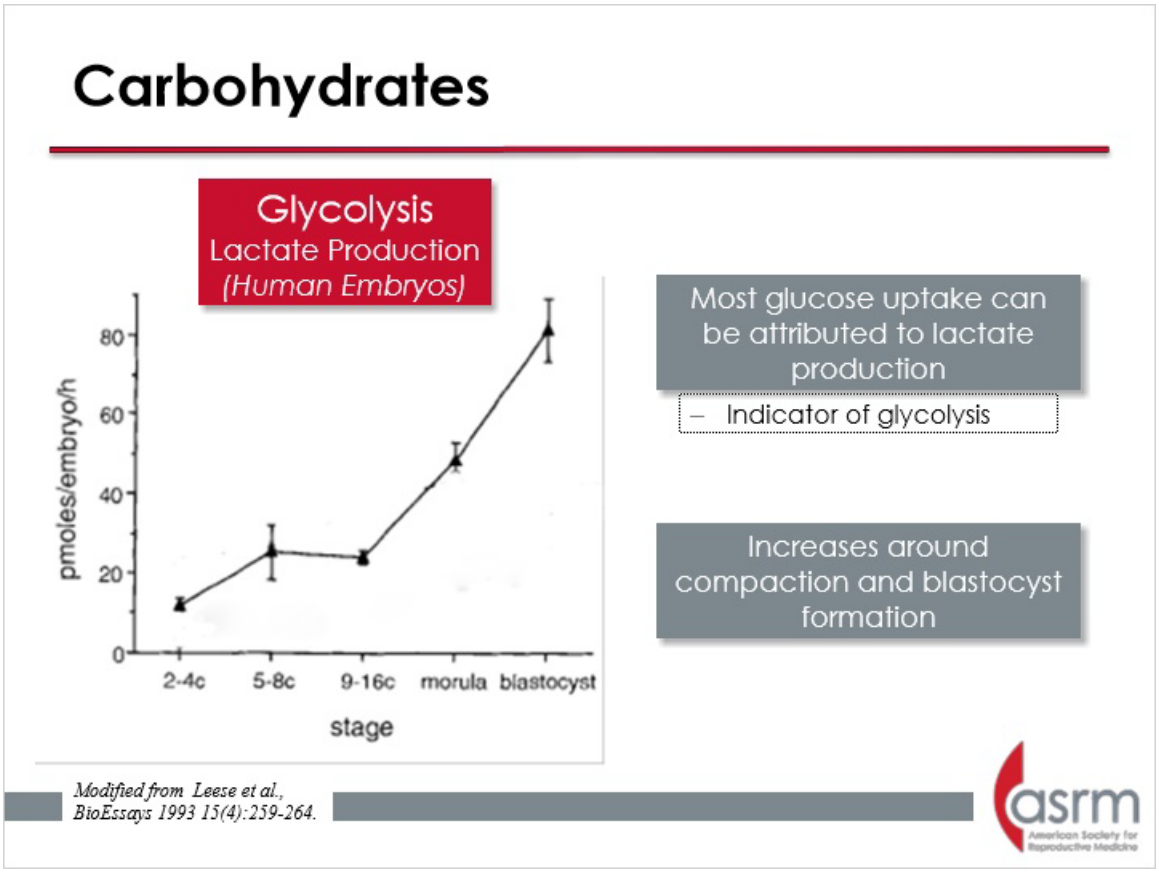
1.17 Carbohydrates



Notes:

Interestingly, studies indicate that glucose uptake may indicate embryo quality, as higher grade blastocysts and embryos leading to pregnancy had greater glucose uptake compared with poorer quality blastocysts or embryos not resulting in pregnancy. Unfortunately, use of this assay has not received widespread clinical application as a selective tool.

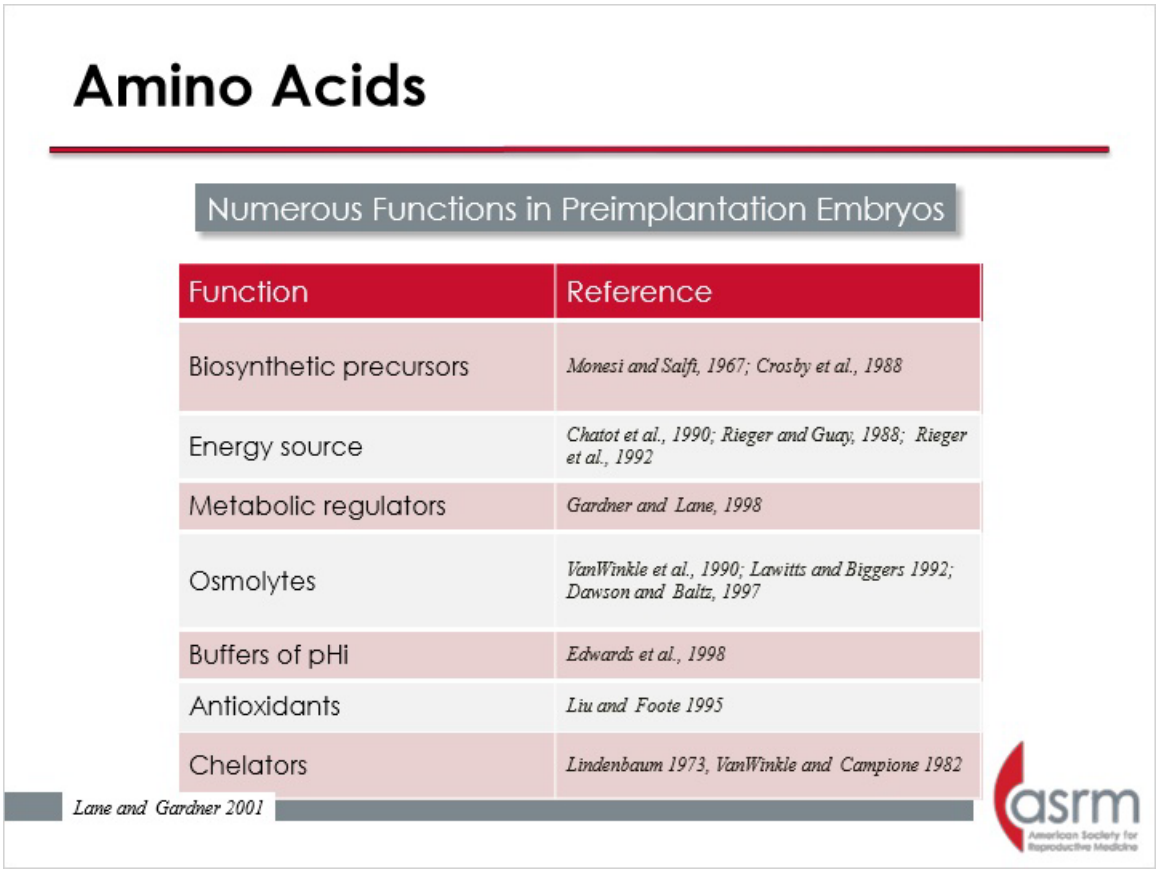
1.18 Carbohydrates



Notes:

The majority of glucose metabolism is via glycolysis, which, as mentioned, increases around compaction and blastocyst formation.

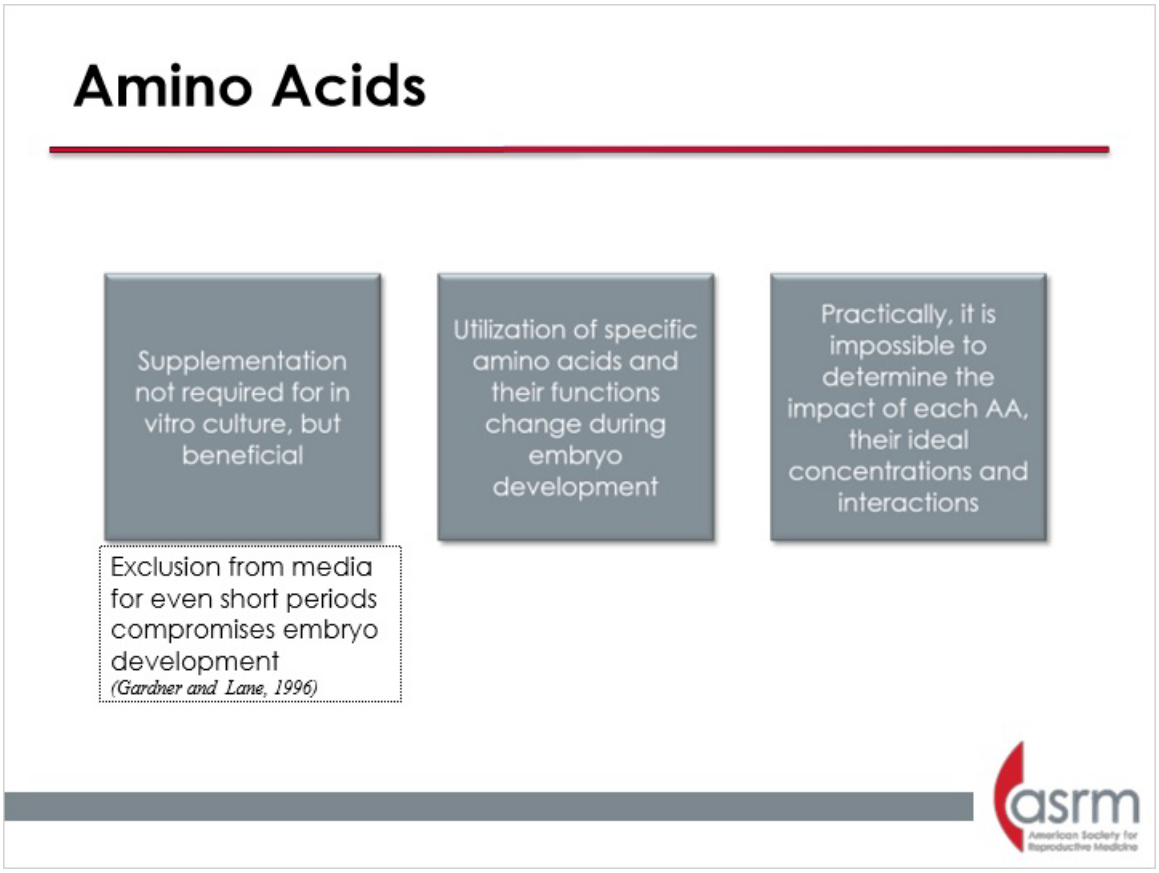
1.19 Amino Acids



Notes:

The inclusion of amino acids is an area that has received much attention and has proven especially important for development of modern embryo culture media. Amino acids have been shown to fulfill numerous physiologic roles in the preimplantation embryo, including biosynthetic precursors, energy substrates, metabolic regulators, osmolytes, pH buffers, antioxidants, and chelators. There are numerous other publications that indicate this extensive role.

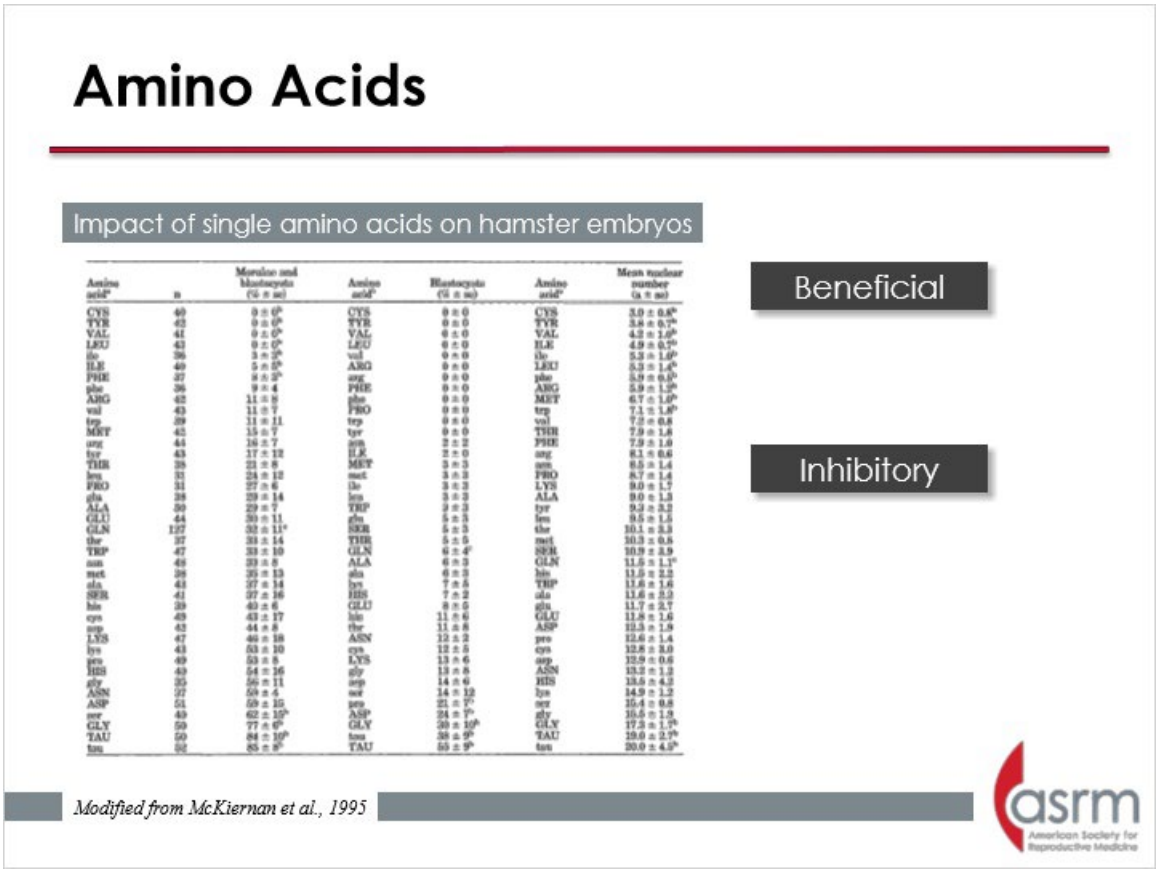
1.20 Amino Acids



Notes:

Importantly, amino acids are not necessarily required for in vitro embryo culture. Indeed, early media such as HTF did not contain amino acids supplements; however, their inclusion has been shown to be beneficial. One study indicated that their exclusion from media during even short periods of mouse embryo handling compromised development. Thus, all modern clinical embryo culture media contain some assortment of amino acids. Importantly, their use and specific function change during development and, practically, it is impossible to determine the impact of each individual amino acid or to determine their ideal concentration or their interactions.

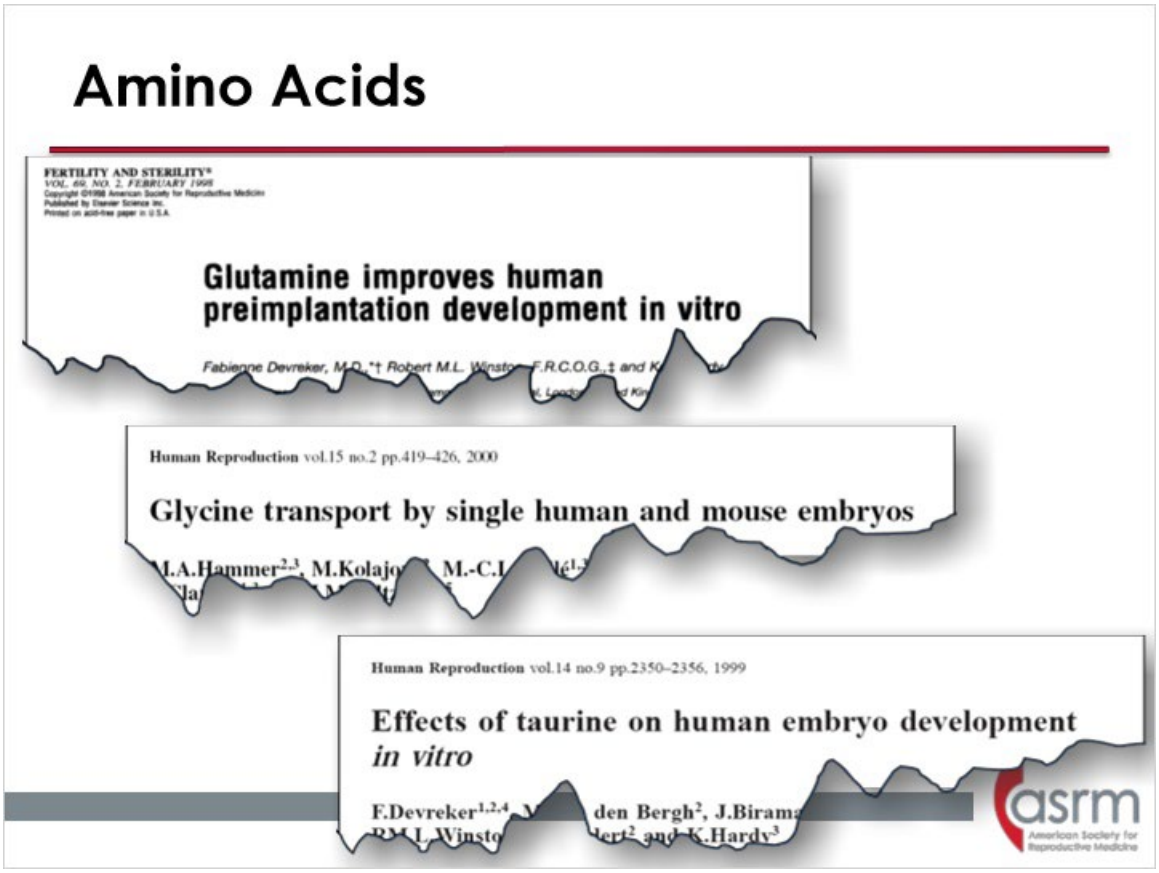
1.21 Amino Acids



Notes:

An early study using 1-cell hamster embryos examined the impact of various individual amino acids and their concentrations on development. While the goal is not to analyze this entire table, and there were several other experiments in the paper, it is meant to show that attempts have been made to analyze individual amino acids. While this study just looked at high and low concentrations, and did not do exhaustive studies or examine all the interactive effects, it was important because it did start to indicate that some amino acids, such as glycine, taurine and glutamine, were beneficial for development, while others were found to be inhibitory at varying concentrations.

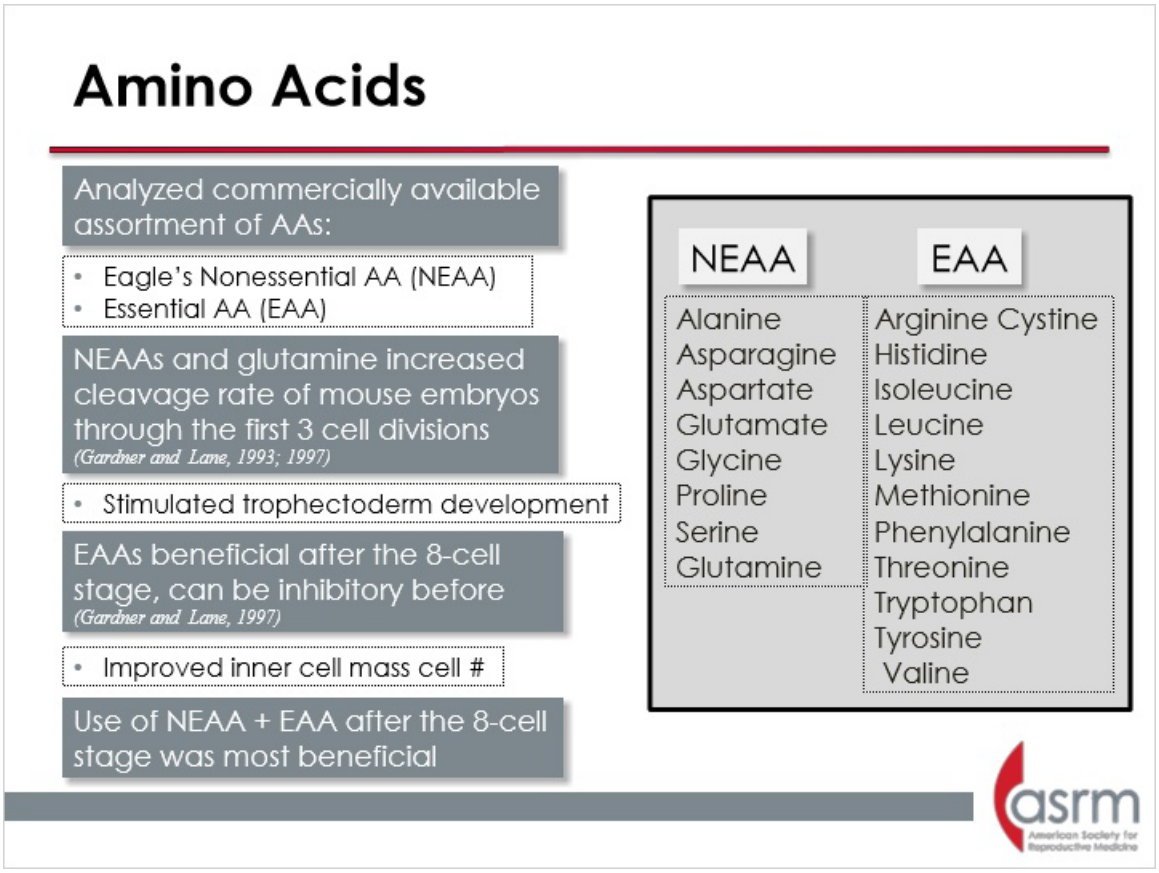
1.22 Amino Acids



Notes:

Interestingly, the three most beneficial amino acids found for hamster embryos have also been found to be beneficial for human embryos.

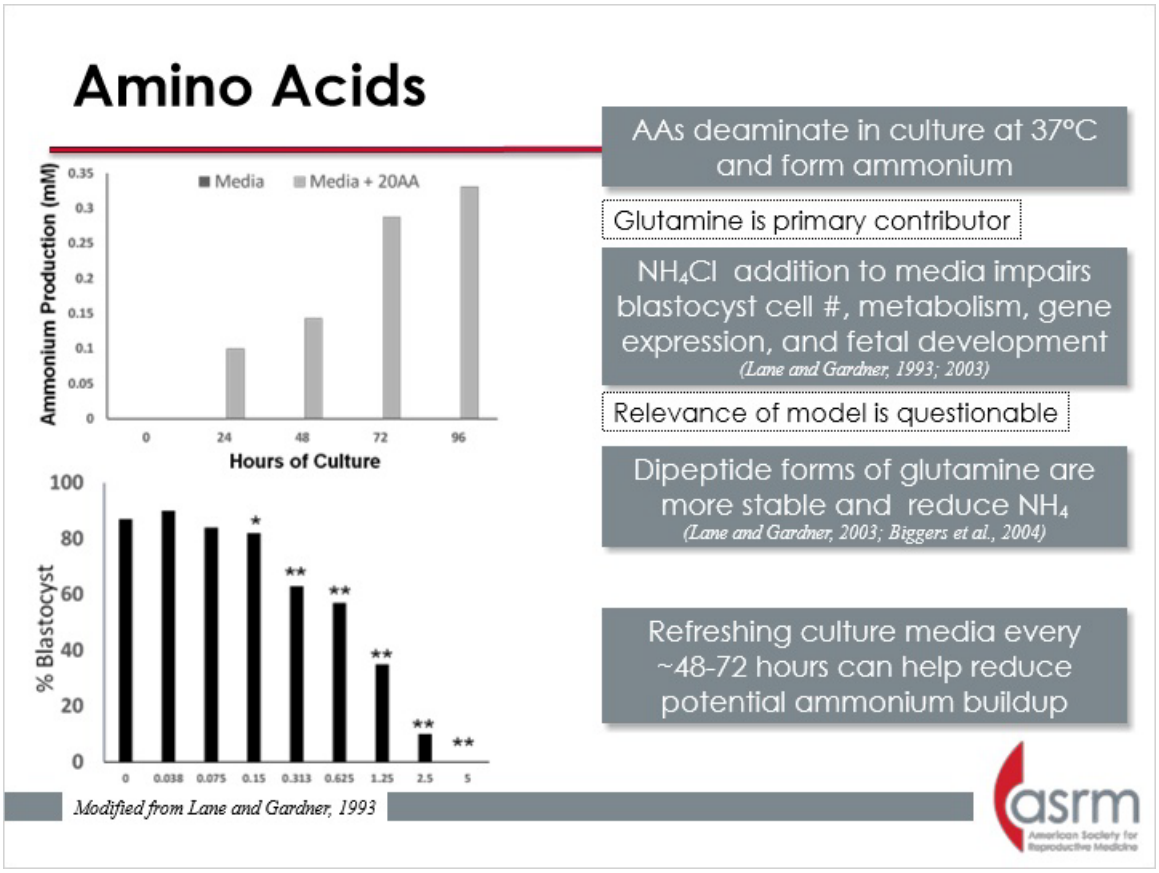
1.23 Amino Acids



Notes:

Two well-known studies used a different approach to analyze the impact of amino acids during embryo culture. This approach used commercially available mixtures of amino acids, Eagle's nonessential (NEAA) and essential (EAA). Essential amino acids are classically described as those that cannot be synthesized de novo and must be obtained in the diet, while nonessential can be synthesized. However, these are also classified by commercial naming and the assortment varies from the classical definition. These commercially available amino acids were used to culture mouse embryos, and it was shown that NEAAs and glutamine were beneficial during cleavage-stage embryo development, and also appeared to increase trophectoderm development. Conversely, essential amino acids were inhibitory during early cleavage and beneficial after the 8-cell stage during compaction and blastocyst formation. EAAs appeared to stimulate development of the inner cell mass. Use of both NEAA and EAAs were most beneficial following the 8-cell stage. It should be noted that concentrations of the individual amino acids were not heavily explored, as they were simply supplied at the amounts determined in the commercial mixture.

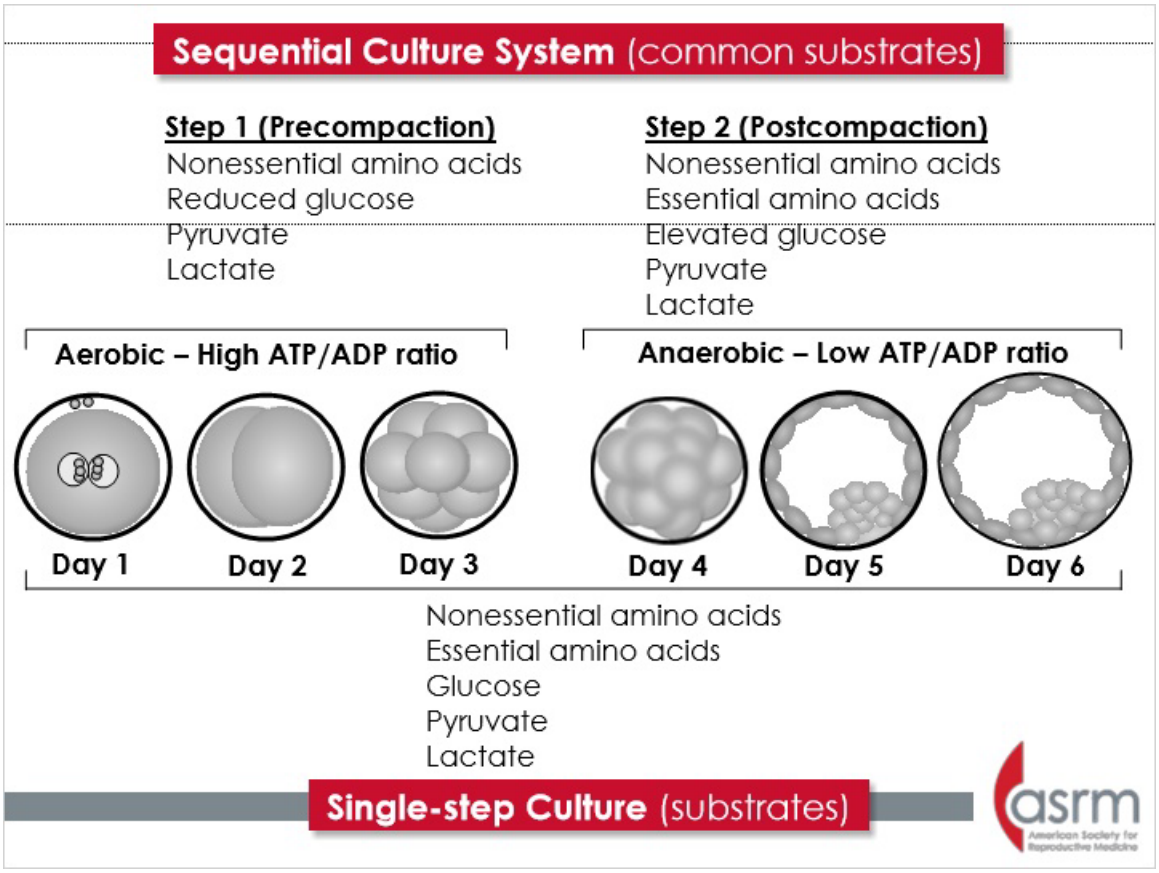
1.24 Amino Acids



Notes:

Caution must be used with inclusion of amino acids in embryo culture media. Amino acids can deaminate at elevated temperatures and form ammonia. The main contributor to ammonium production is glutamine. Ammonium is detrimental and has been shown to impact blastocyst development and metabolism. In addition, ammonium in culture is reported to lead to exencephaly in mouse offspring. It should be noted, however, that the relevance of this finding is questionable, as it could be due to nonphysiologic levels of amino acids used in the Eagle's formulations. Many media now use lower amino acid concentrations. Furthermore, use of dipeptide forms of amino acids are more stable in culture and do not form high levels of ammonium and can support embryo development. Thus, the issue of ammonia has been reduced greatly, if not removed. Alternatively, culture media can also be changed at regular intervals to remove any ammonium. This can be done with a sequential or a single-step media system.

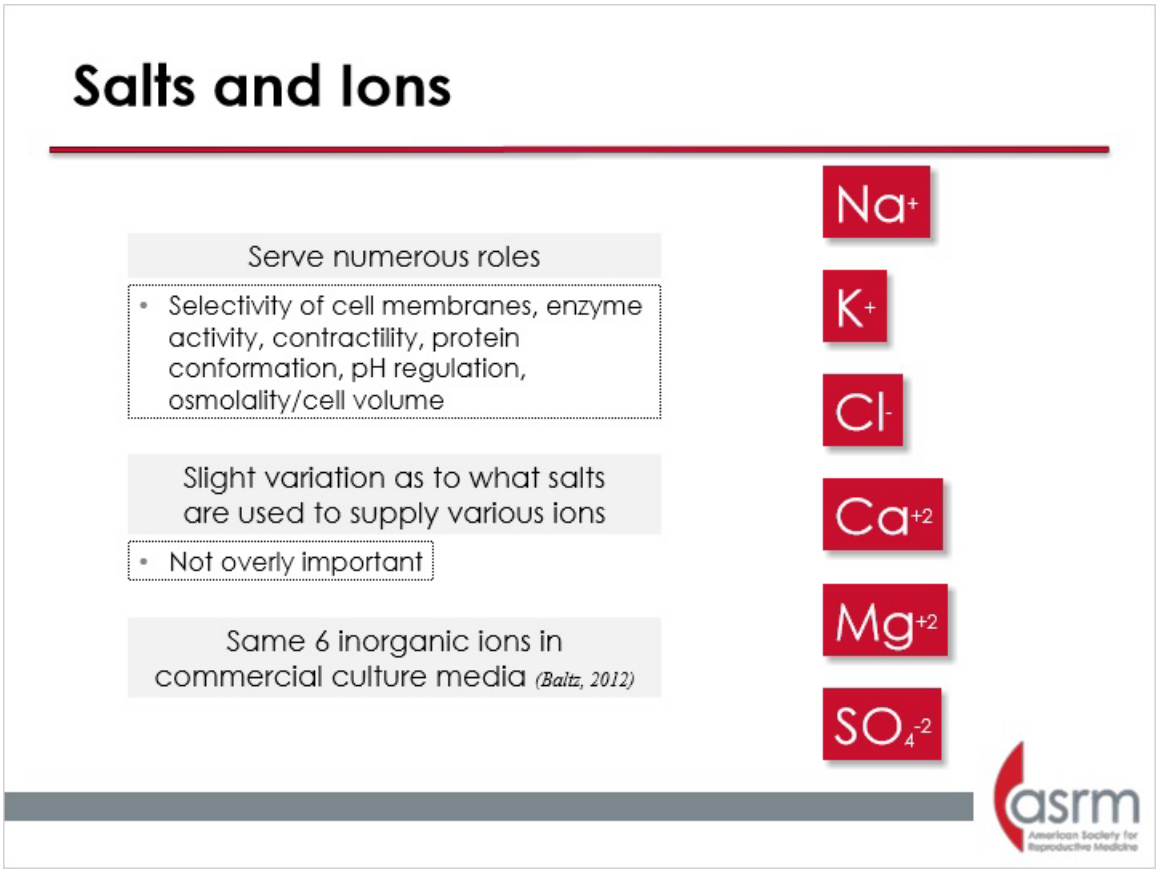
1.25 Sequential Culture System



Notes:

As a result of these studies examining the impact of energy substrates, common embryo culture media tend to follow the formulations seen here, though specific concentrations of these substrates will vary.

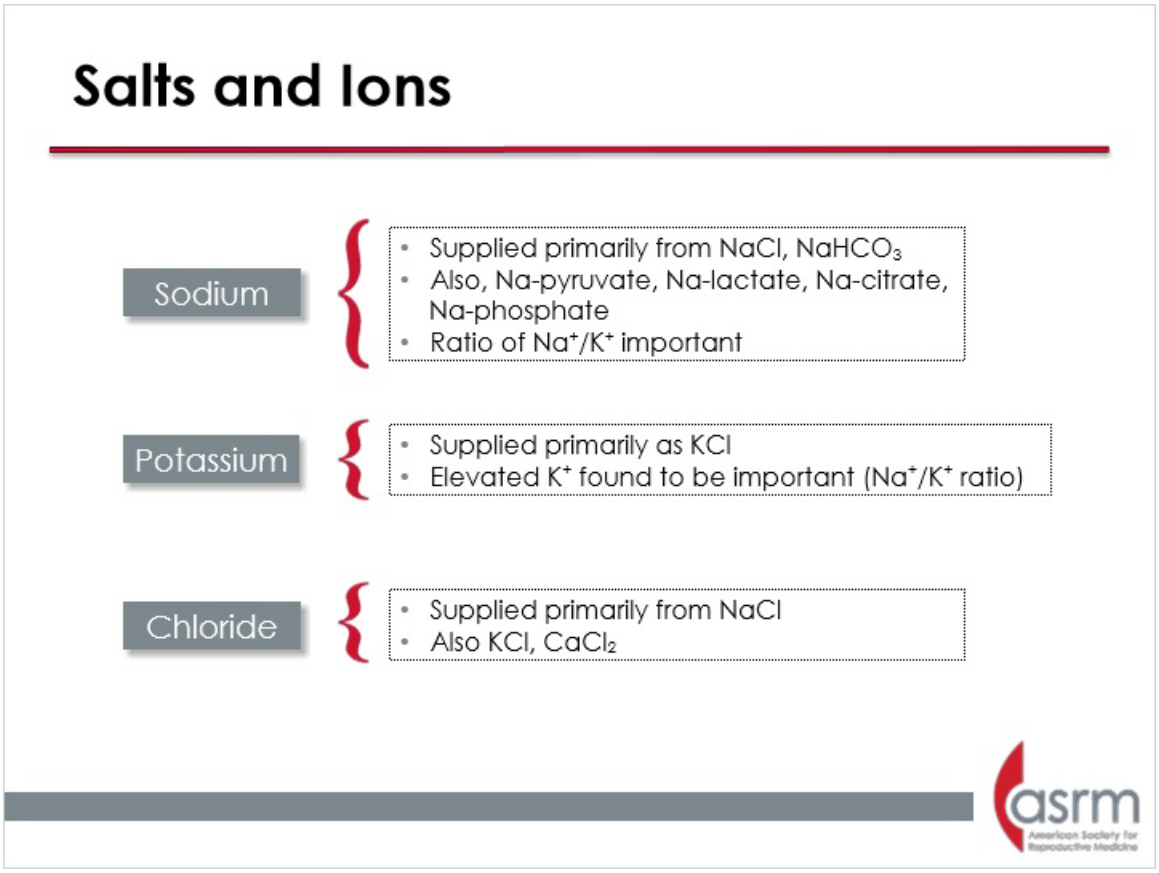
1.26 Salts and Ions



Notes:

Another important area of modern embryo culture media is the salt and ion composition. These factors are often taken for granted, but play instrumental roles in regulator cell membranes, enzyme activity, and cell regulation of pH and volume. There are slight variations among commercial media as to what salts are used to supply the various ions, but this is not overly important, as these dissociate in solution and it is the ions themselves and their concentrations that are important. Despite these minor differences, all commercial embryo culture media contain the same 6 inorganic ions, sodium, potassium, chloride, calcium, magnesium, and sulfate.

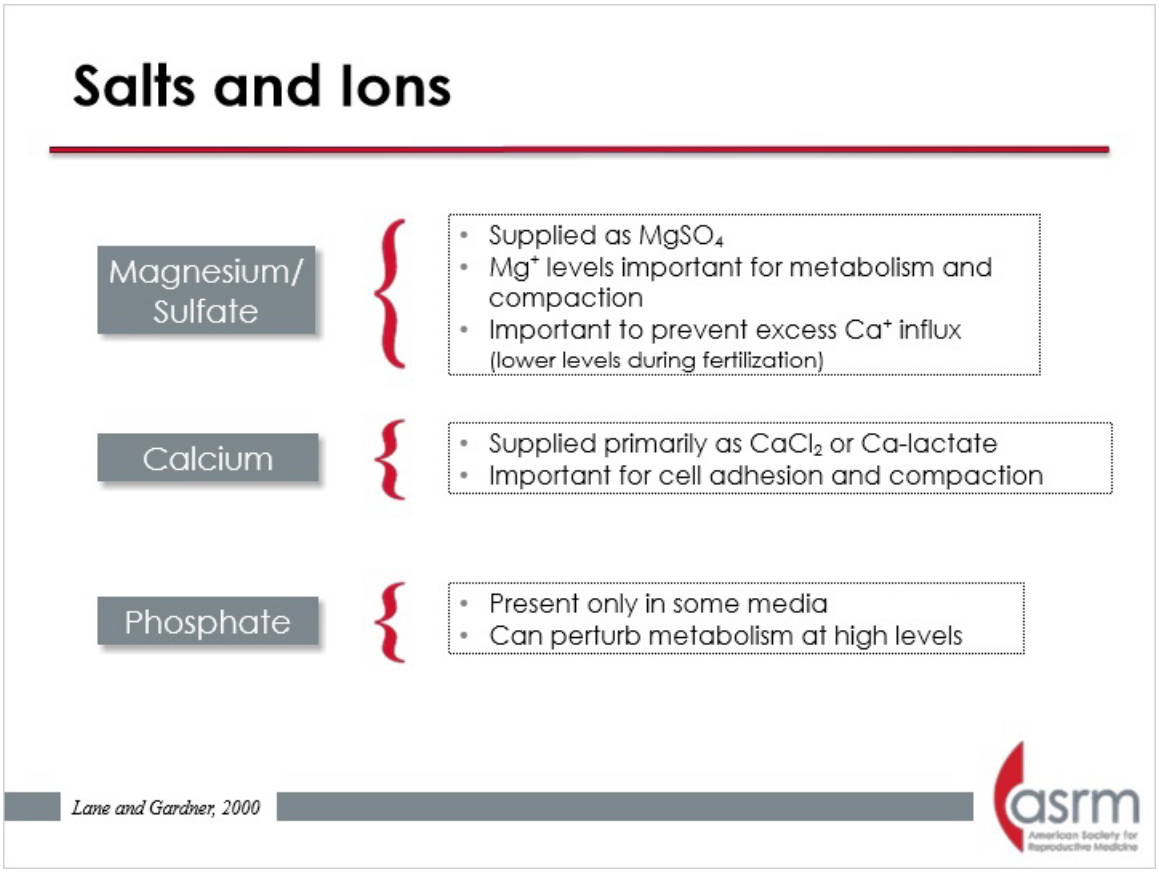
1.27 Salts and Ions



Notes:

Sodium is primarily supplied by sodium chloride and sodium bicarbonate. Sodium can also be added via salts of pyruvate, lactate, citrate, and others. Importantly, it is not so much the amount of sodium itself that appears important, but rather the ratio of sodium to potassium. Potassium is supplied primarily from potassium chloride. As mentioned, the ratio of this ion is important, as noted by the development of HTF, as well as potassium simplex optimized medium (KSOM). This likely has to do, in part, with operation of the sodium/potassium adenosine triphosphatase (ATPase). Chloride ions are supplied primarily from sodium chloride, but also potassium chloride and calcium chloride.

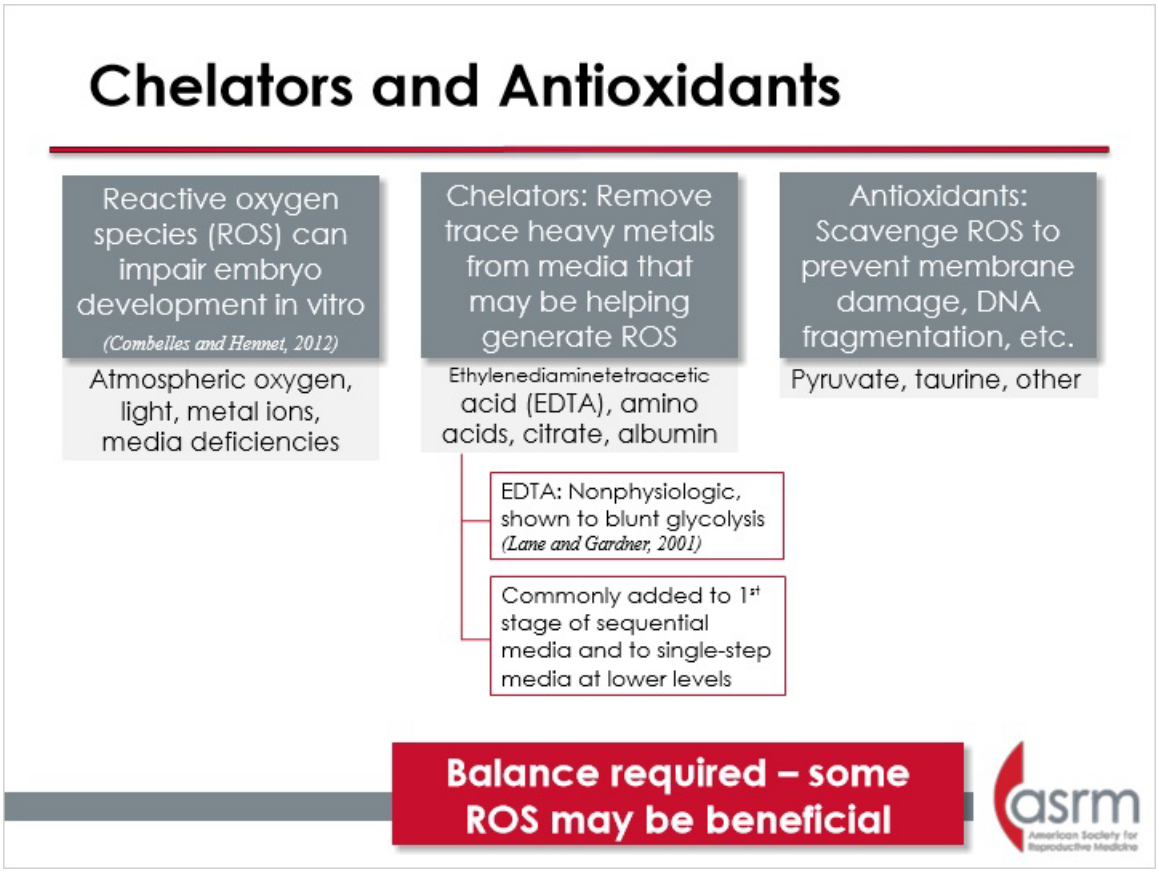
1.28 Salts and Ions



Notes:

Magnesium and sulfate are supplied as magnesium sulfate. Magnesium levels are important for regulating enzymes involved in glucose metabolism as well as regulating cell adhesion during compaction via E-cadherin. Furthermore, magnesium helps prevent excess uptake of calcium from the media via calcium channels, which could perturb intracellular homeostasis. As a result, some fertilization media contain lower levels of magnesium compared with embryo culture media. Calcium is supplied either from calcium chloride or calcium lactate. Calcium is also important for cell adhesion and compaction, which is a prerequisite for blastocyst development. Finally, phosphate is present only in some embryo culture media. At high levels, it can perturb glucose metabolism.

1.29 Chelators and Antioxidants

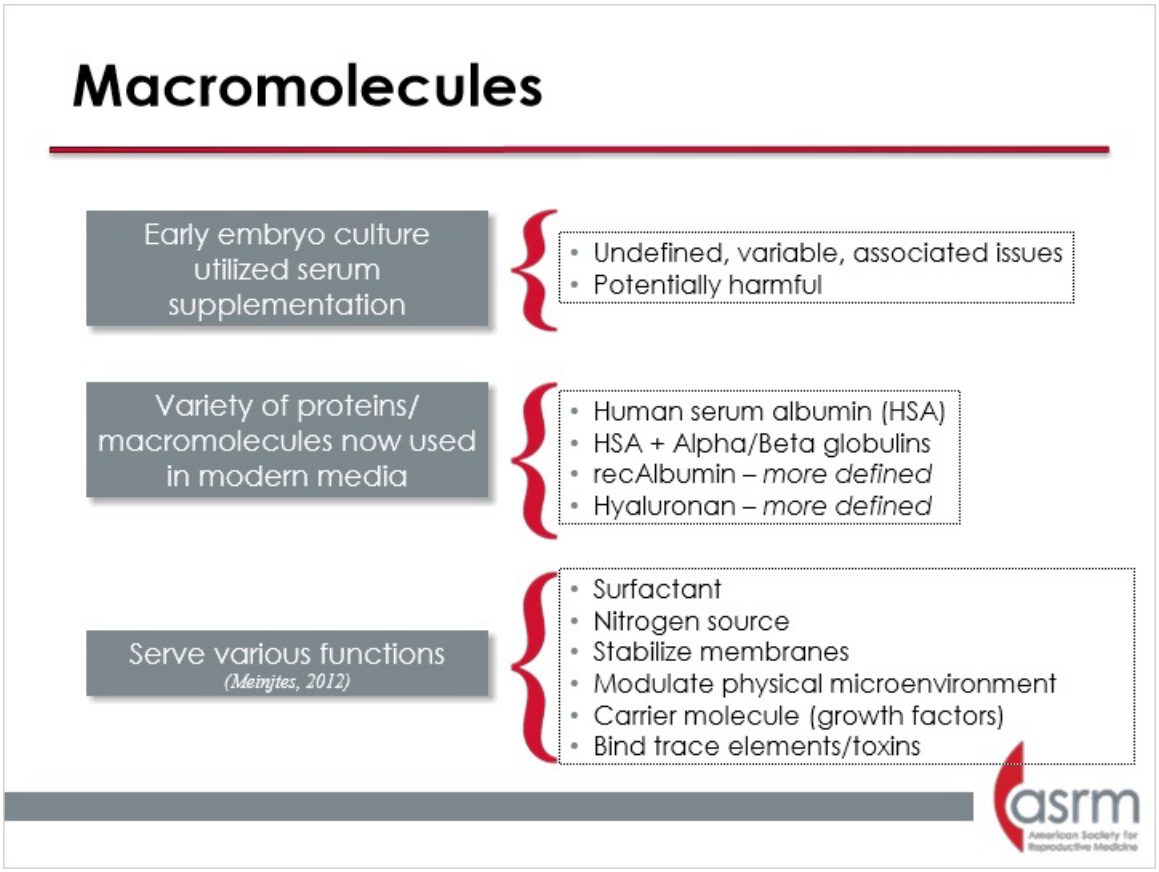


Notes:

Chelators and antioxidants in culture media primarily serve to combat reactive oxygen species (ROS). ROS can impair embryo development in vitro. Their formation is hastened via use of atmospheric oxygen levels, excess light exposure, or heavy metal ions in culture, or they may be due to media deficiencies. Fortunately, these ROS can be combated to some extent via media supplementation. Chelators serve to remove trace heavy metals from media that may lead to generation of ROS. Common chelators in media include ethylenediaminetetraacetic acid (EDTA), various amino acids, and citrate. Notably, EDTA is not a physiologic supplement and is not found in vivo as some of the others are. Thus, this chelator tends to garner a lot of attention. Studies have shown that elevated levels of EDTA can be detrimental and that it blunts glycolysis. Thus, it is usually added to just the first portion of a sequential culture system or at low levels in single-step media. With other physiologic chelators present and depending on the culture system, there is discussion about whether EDTA should be included in media or not. Other additives to help combat ROS include antioxidants. These help to scavenge free oxygen radicals to help prevent cell damage. Common antioxidants in culture media include pyruvate and some amino acids, like taurine. There are other antioxidants, including some enzymes and vitamins, but these are not commonly added to most

commercial media. It should be noted that too much of a good thing can be bad. Balance is required when it comes to antioxidants and chelators. Some ROS can be instrumental in cell signaling, and thus some level may be beneficial. The idea is to prevent excess or harmful levels of ROS, not to deplete them completely.

1.30 Macromolecules

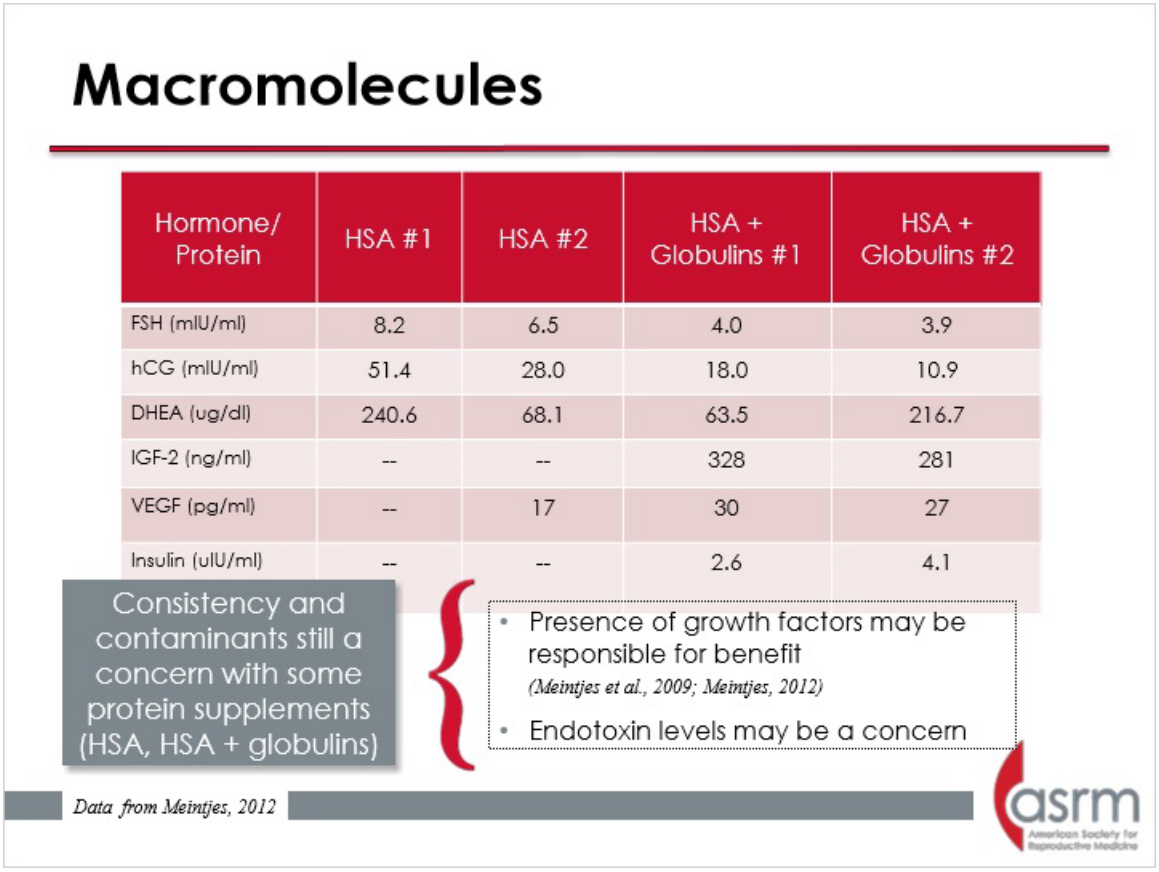


Notes:

Another very important component of embryo culture media includes macromolecule supplementation. All media are supplemented with some sort of macromolecule. Early media utilized serum preparations, which is problematic due to the undefined nature of serum, its variability, and associated issues. In practice, this meant that there was also great variability between batches, which was problematic for repeatability. Also, use of serum has been shown to perturb mitochondrial function and is well known as a culprit for large-offspring syndrome in cattle. Thus, moving away from its use was essential in improving culture media. Currently, a variety of more defined macromolecules are used in commercial media. These include HSA, recombinant albumin, complex supplements of HSA with alpha/beta globulins, as well as hyaluronan. These serve various functions,

including acting as surfactant to prevent cell sticking. As a nitrogen source for metabolism they may help stabilize membranes, but they also act as carrier molecules, including growth factors or toxins, and may also alter the physical environment.

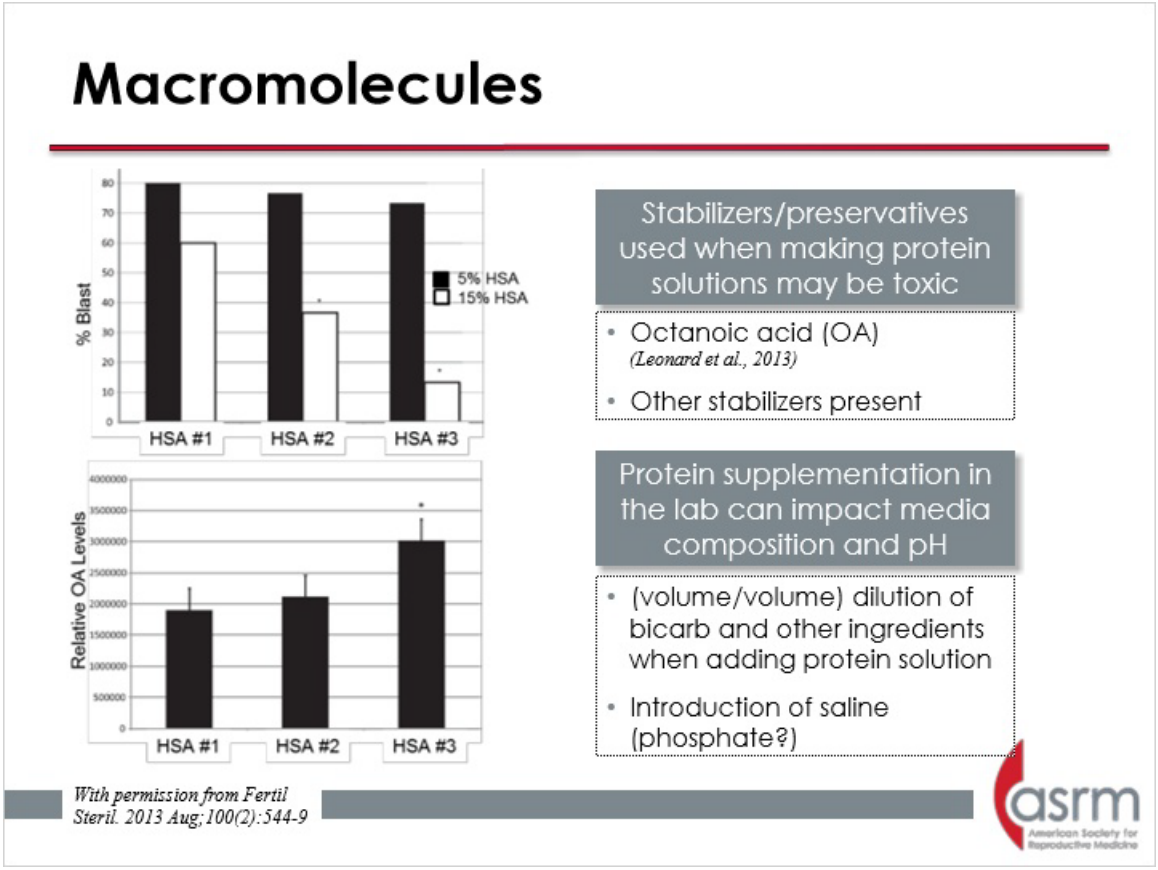
1.31 Macromolecules



Notes:

There are some problems with macromolecule supplementation. Protein supplements are one of the less well-defined components of the culture system. As mentioned, proteins like albumin can bind other molecules. Thus, when these proteins are precipitated from serum or plasma fractions, they often pull down other components. Therefore, consistency between preparations and possible contaminants can result in variability. Endotoxin levels may also be problematic and can impair embryo development. An example of some of the variability that occurs between protein preparations can be seen in this table. This is a subset of data from a lab that examined preparations of HSA and HSA with globulins. Note that levels of "contaminants" or various hormones and growth factors can vary significantly between lots. This may help explain why some protein supplements function better or worse than others.

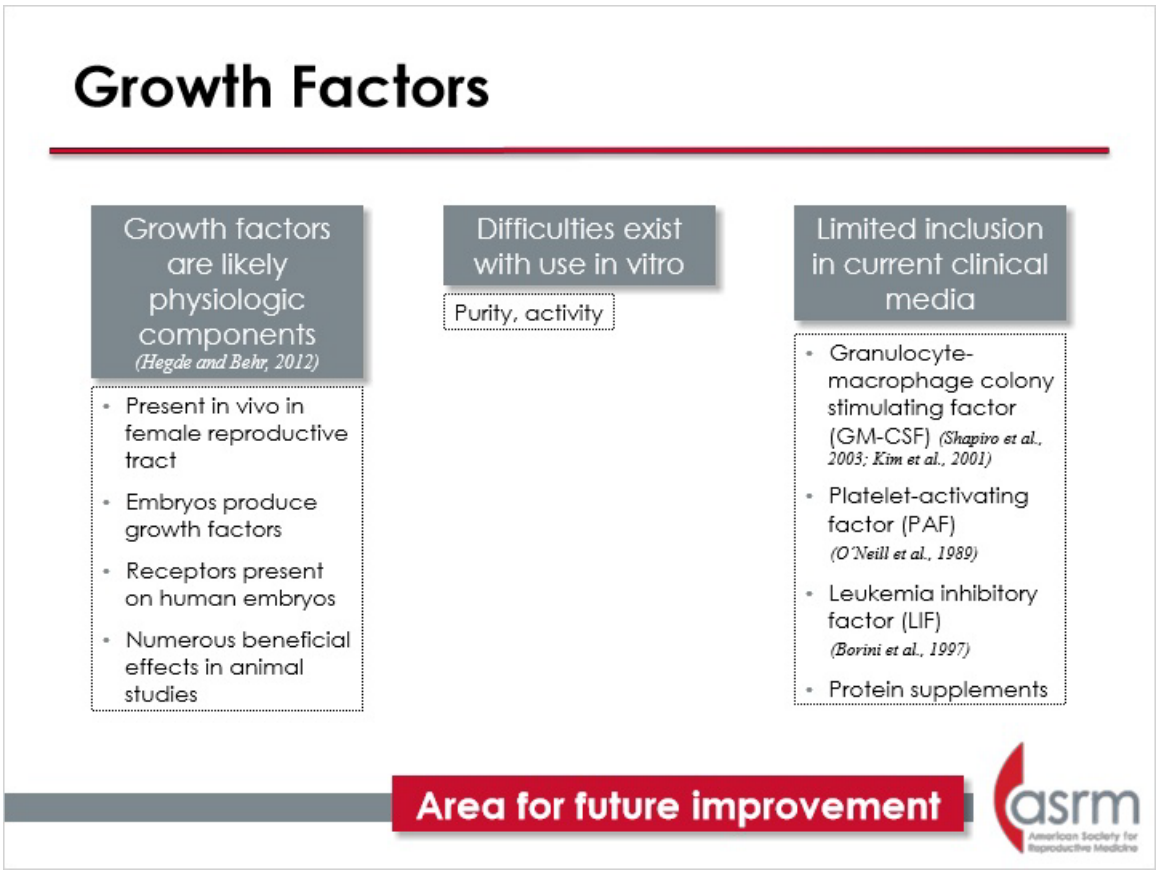
1.32 Macromolecules



Notes:

In addition to the protein precipitation methods and the variability of their components, the preparation of the protein solutions may also impact their efficacy. Various stabilizers and preservatives are added to a saline solution when making solutions of HSA. These preservatives can themselves be toxic. A recent study showed variability in mouse blastocyst development when using different lots and concentrations of HSA. High protein concentrations of 15% of lots #2 and #3 reduced embryo development. These poorly performing lots of protein had higher levels of octanoic acid than the high-performing protein in lot #1. Furthermore, when adding solutions of protein in the lab, such as 10% or 20%, this dilutes the culture media by 10% or 20% volume/volume. This can impact pH and concentrations of other media components, possibly impacting media efficacy. While having media presupplemented with protein by the manufacturer can avoid some of the dilution issues, it gives the individual lab less control over the protein lot/product used. While convenient, recall that there is immense variability in protein solutions, so this may or may not be worth the tradeoff.

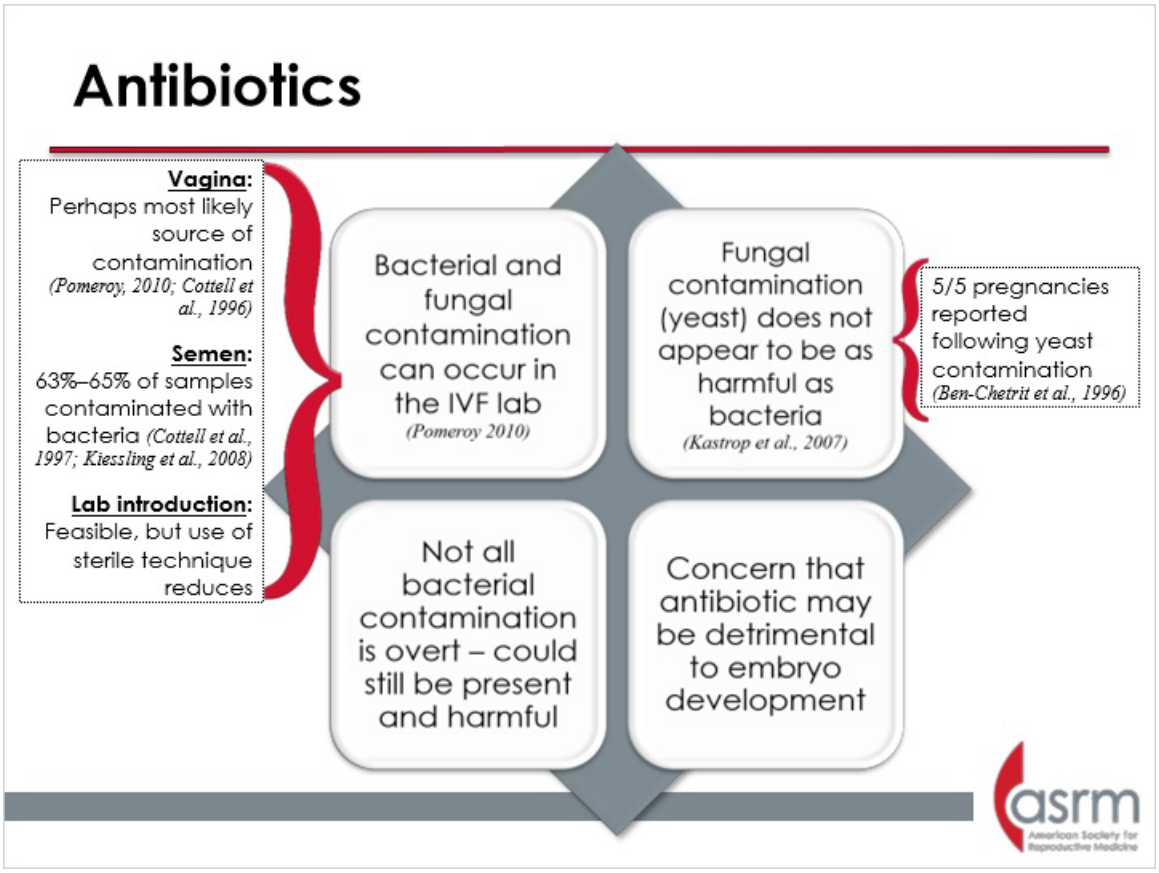
1.33 Growth Factors



Notes:

Growth factors are a controversial topic with respect to culture media supplementation. These factors are undoubtedly physiologic, as they have been identified in the female reproductive tract. Embryos have been shown to produce various growth factors, and receptors for several growth factors have been found on gametes and embryos. However, difficulties do exist with their inclusion in media. These stem from purity issues. Also, not all growth factors will be beneficial. As a result, though numerous animal studies indicate a benefit of growth factor supplementation, there have been very limited reports of their use in human IVF. There are reports of benefit of granulocyte-macrophage colony stimulating factor (GM-CSF), platelet-activating factor (PAF), and leukemia inhibitory factor (LIF) and the already mentioned growth factor "contamination" from some protein supplements. Thus, this is likely an area of future improvement for embryo culture.

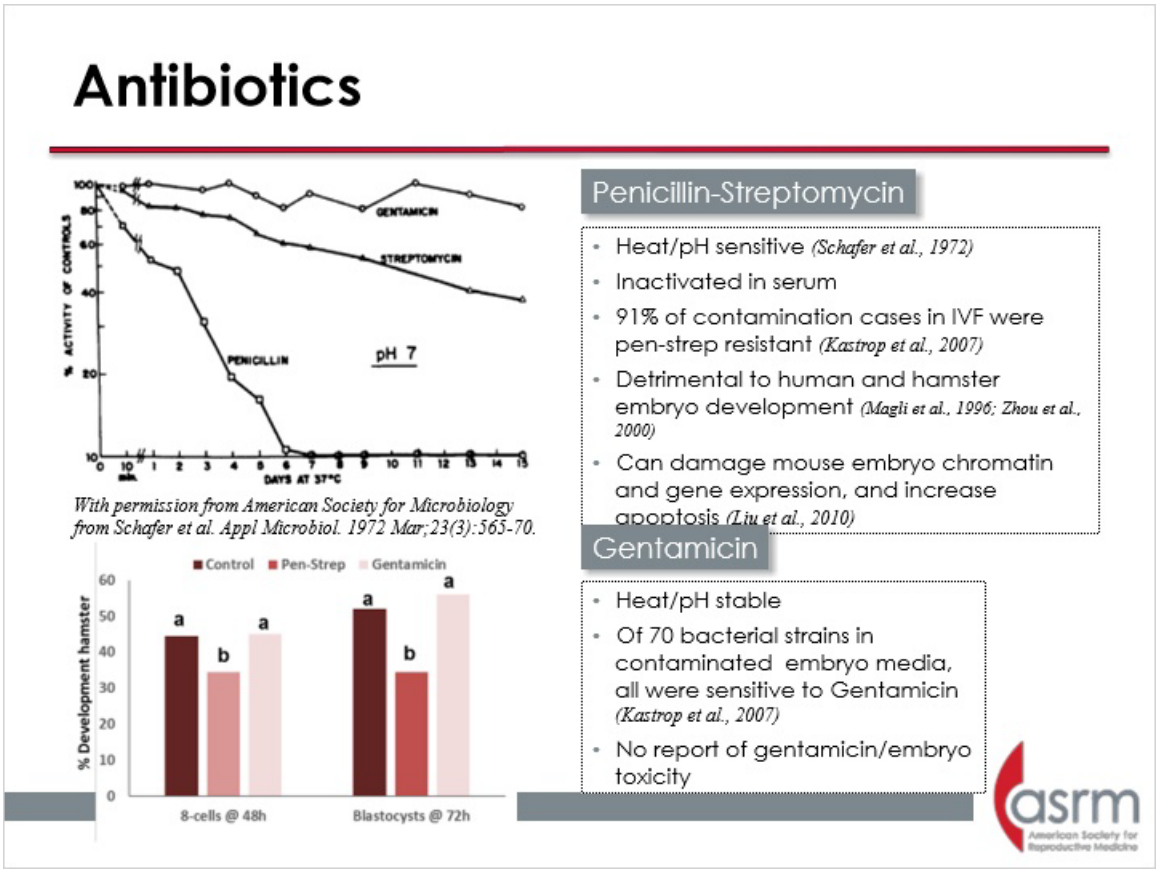
1.34 Antibiotics



Notes:

Another area of potential controversy is the use of antibiotics in culture media. As is well known, contamination can occur in the laboratory. Recall that neither the vagina nor semen are sterile and are often contaminated; the risk is very real. Though the lab can introduce contamination itself, use of sterile technique greatly reduces this change. It appears that of the two main types of contamination, fungal contamination is less harmful than bacterial. Pregnancies have been reported in cases of fungal contamination. However, bacterial contamination is a bit more severe. Also, not all contamination is overt. Thus, antibiotics are often included in media. However, there is some concern that these could actually be detrimental to embryo development.

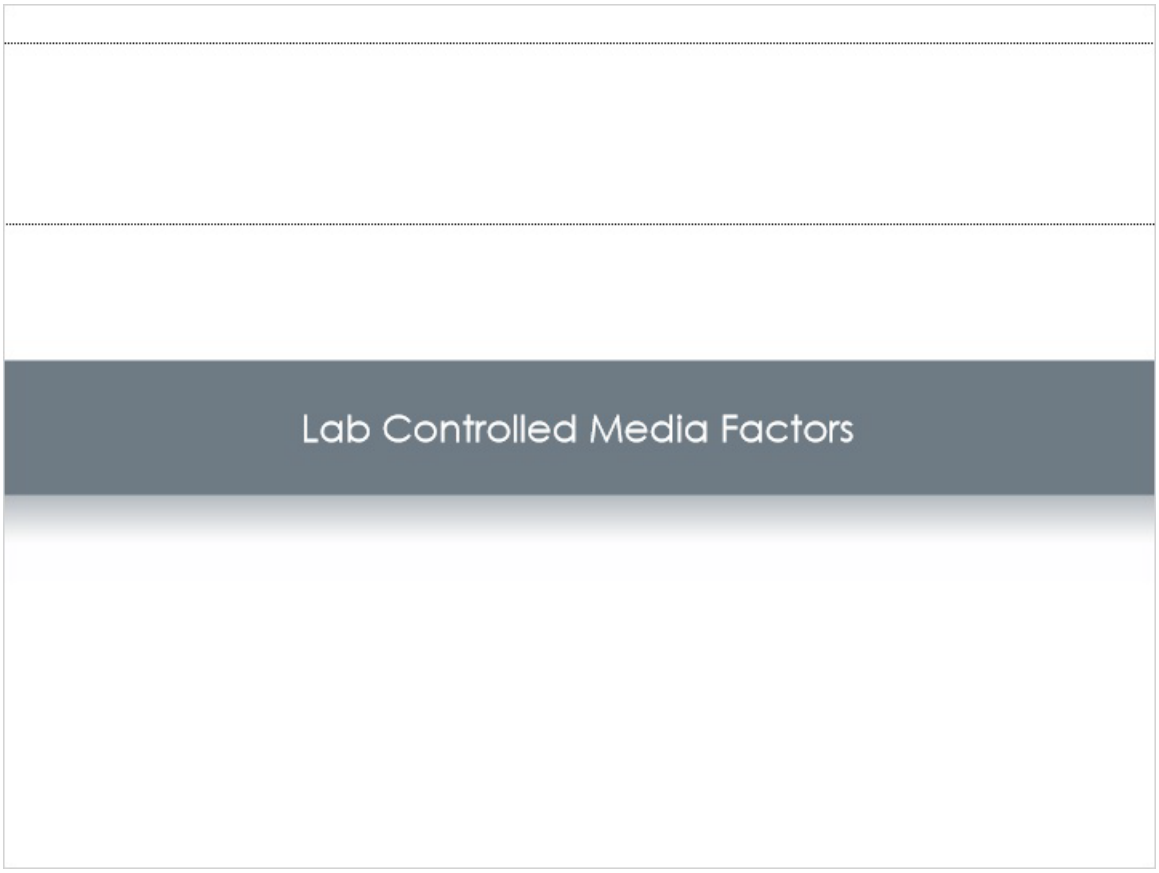
1.35 Antibiotics



Notes:

Penicillin and streptomycin are two common antibiotics that are often placed into the same cocktail and referred to as “pen-strep.” Unfortunately, both antibiotics are heat and pH sensitive, with rapidly decreasing activity at 37 degrees Celsius at pH 7. Furthermore, in studying contamination during IVF, it was found that 91% of cases were pen-strep resistant. In addition, the antibiotic cocktail was found to be detrimental to hamster embryo development, as seen in the lower graph. It also has been shown to be damaging to mouse embryo chromatin and gene expression, and increases apoptosis. Conversely, gentamicin is heat and pH stable, was active against all 70 strains of bacteria identified in contaminated embryo culture media, and has not been reported to be toxic to embryos. Thus, while theoretically it may be ideal to exclude antibiotics from media, considering the high rate of bacterial and fungal contamination found in semen and follicular aspirates, and considering not all contamination is overt**,** it is likely prudent to include gentamicin in culture media as a preventive measure.

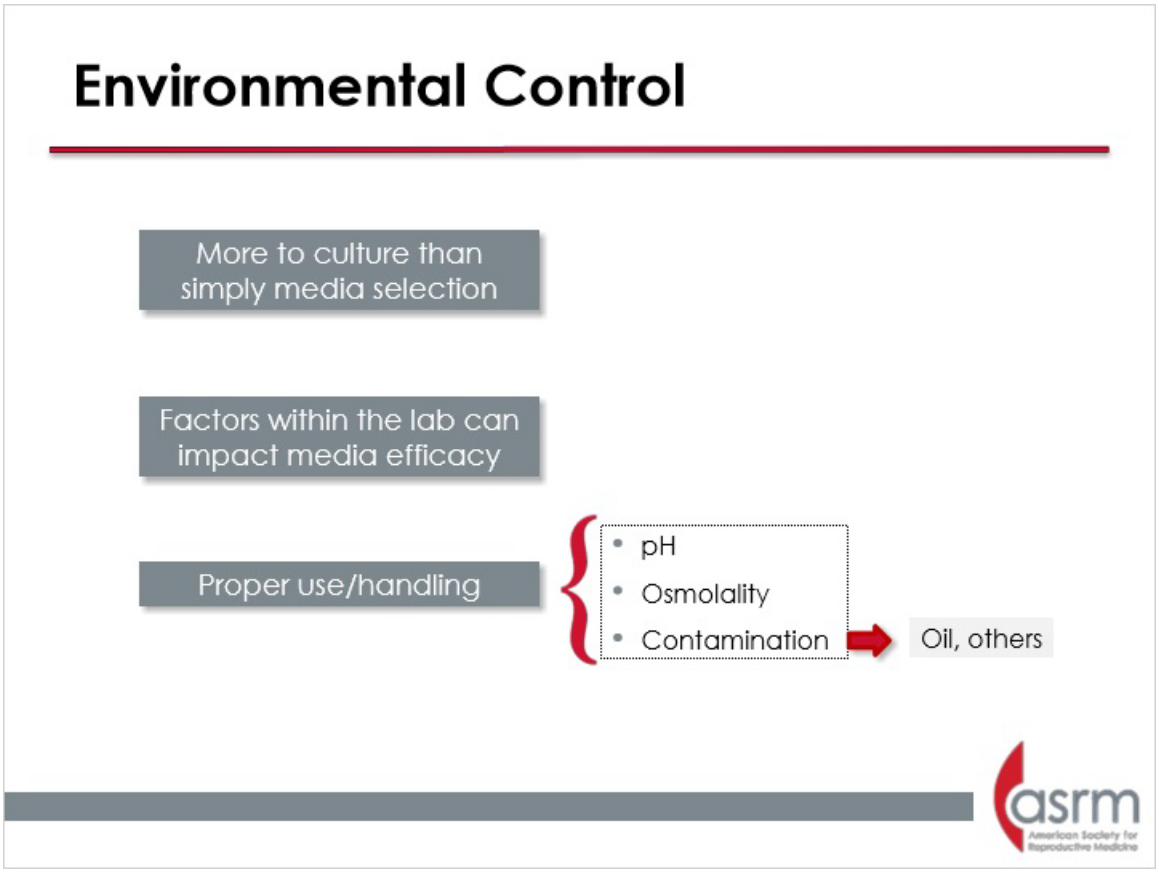
1.36 Lab Controlled Media Factors



Notes:

This section will address laboratory-controlled factors affecting culture media.

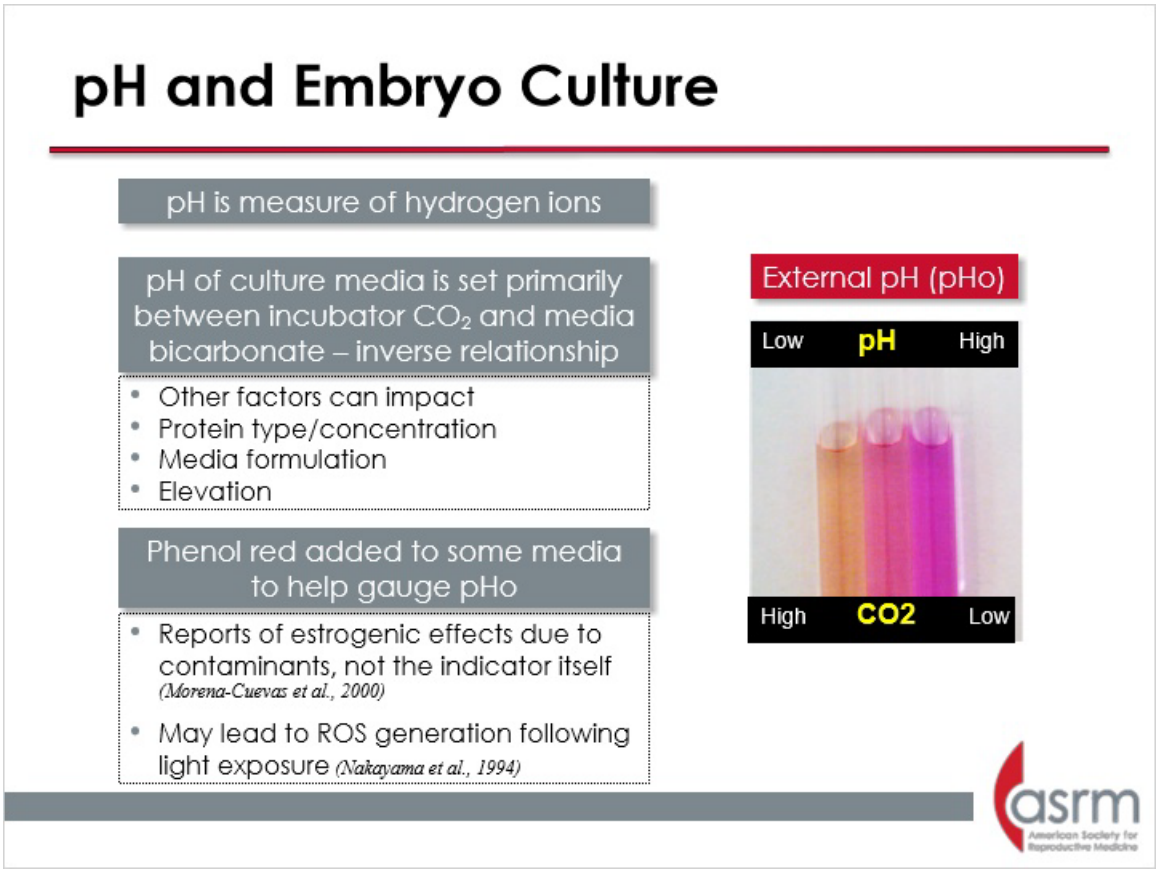
1.37 Environmental Control



Notes:

There is more to embryo culture media than simply selecting a commercial medium. There are factors directly impacted by the laboratory, sometimes unintentionally, that can impact efficacy of the media. Regardless of the medium, proper use and handling are required. Important factors controlled within the lab include media pH and osmolality, as well as contamination from things like mineral oil or other factors (for example, the potential issues with protein supplementation already discussed).

1.38 pH and Embryo Culture

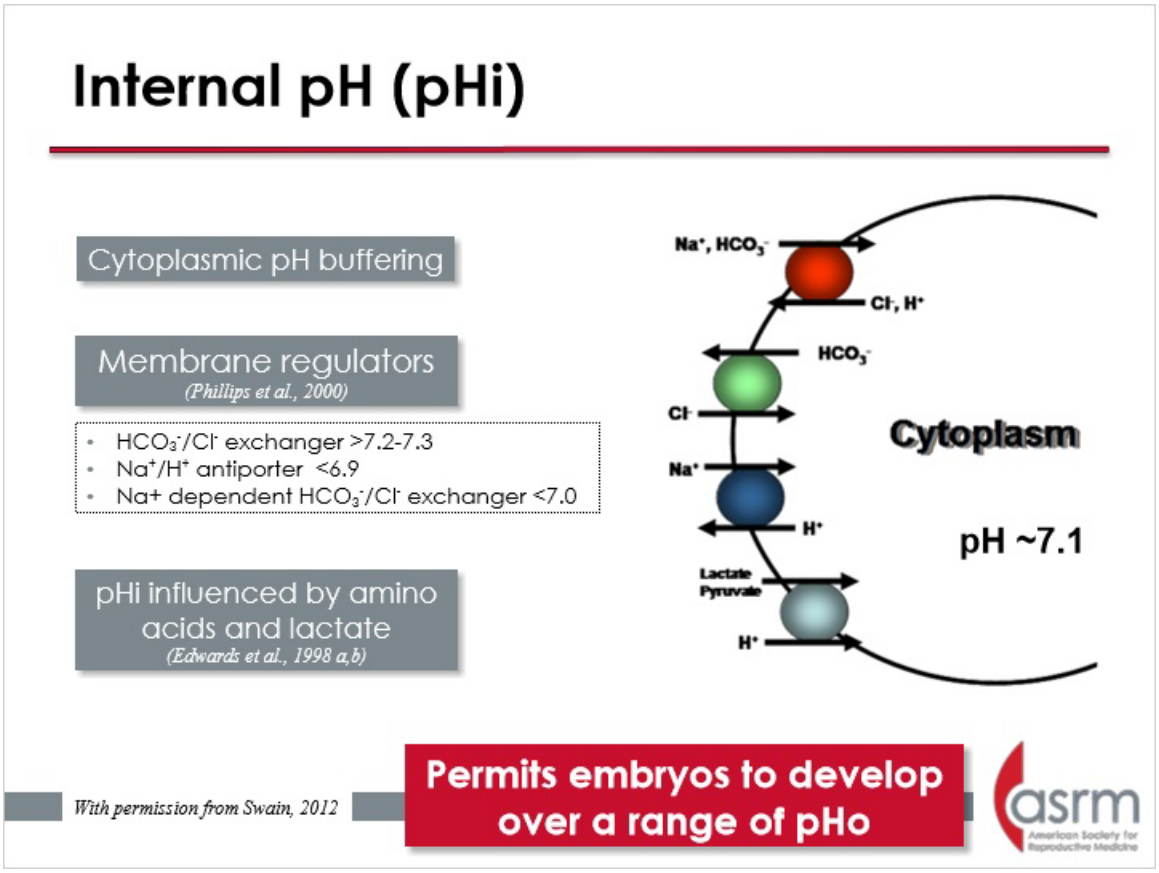


Notes:

pH is perhaps one of the most discussed aspects of culture media that is directly controlled by the individual laboratory. In its most basic definition, pH is the measure of hydrogen ions. This hydrogen ion concentration, or pH, of embryo culture media is primarily set via the interaction of the CO_2 concentrations in the incubator and the sodium bicarbonate concentrations in the media. This is an inverse relationship, with pH decreasing as CO_2 increases. There are also other factors that can impact pH. Protein type and concentration can impact via the acid nature of protein supplements, as well as via dilution of media bicarbonate. Different media can have different compositions, which would require differing CO_2 concentrations to achieve a desired pH. Also, altitude or elevation of a lab could influence how much CO_2 is needed to obtain the desired pH. This begins to show how variable this endpoint can be and illustrates why each lab needs to monitor pH on its own. Some media contain the pH indicator phenol red to help visually gauge pH and act as a sort of safety measure. This can be seen in the image to the right. The pH of the media, often referred to as pHo, can be seen ranging from low to high from left to right. Acidic media is orangish while alkaline media becomes more purple. However, as there was concern over possible toxicity or estrogenic effects of phenol red, some media now exclude this component. Whether this is beneficial or

not is unknown.

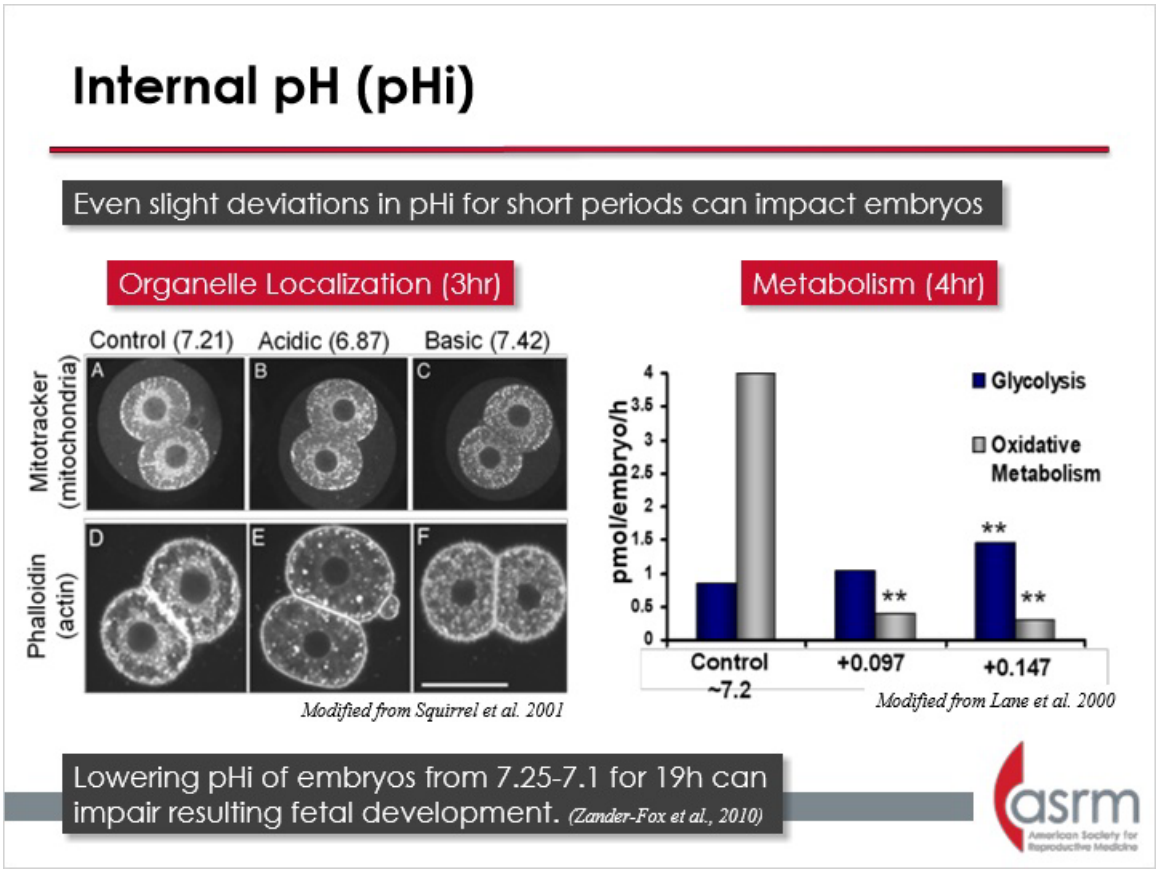
1.39 Internal pH (pHi)



Notes:

The internal pH of the cell, or pHi, is regulated by cytoplasmic proteins, as well as membrane-bound regulators. These regulators activate when certain pH values are reached. Additionally, as noted earlier, pHi of embryos can be impacted by media components, such as amino acids and acids, such as lactate. As a result, the internal pH of human embryos is approximately 7.1. These regulatory mechanisms permit embryos to develop over a range of media pH (pHo). This illustrates embryo plasticity that has been mentioned; however, this can be exceeded and development compromised.

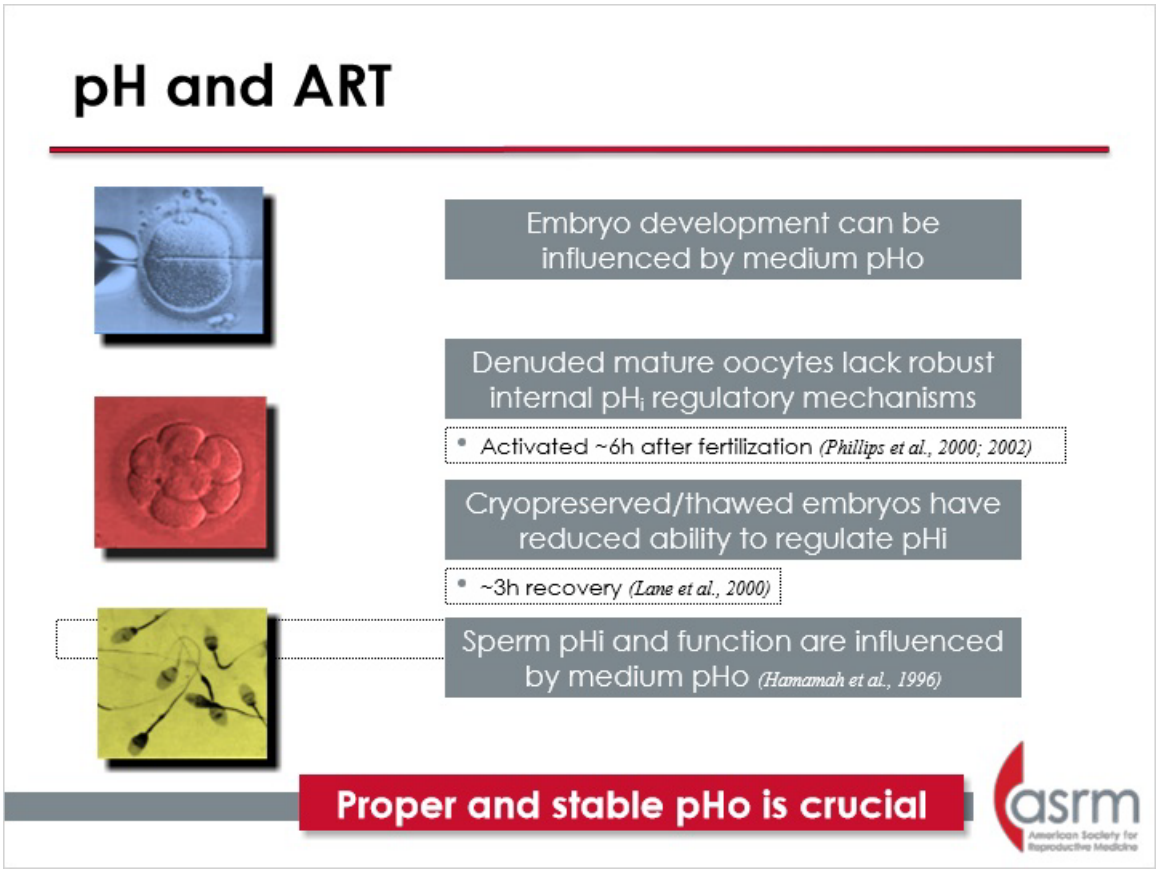
1.40 Internal pH (pHi)



Notes:

Maintenance of pHi is critical to the survival and development of the embryo. Even slight deviations for short periods can have a dramatic impact. For example, slight increases or decreases in hamster embryo pHi for 3 hours resulted in loss of perinuclear localization of mitochondria and actin. Localization of these organelles can have an impact on embryo development. Additionally, slightly raising pH <0.15 units for just 4 hours significantly affects embryo metabolism; recall the correlation between metabolism and embryo quality. Finally, perhaps more concerning is that these deviations in embryo pHi can also impact the resulting fetus.

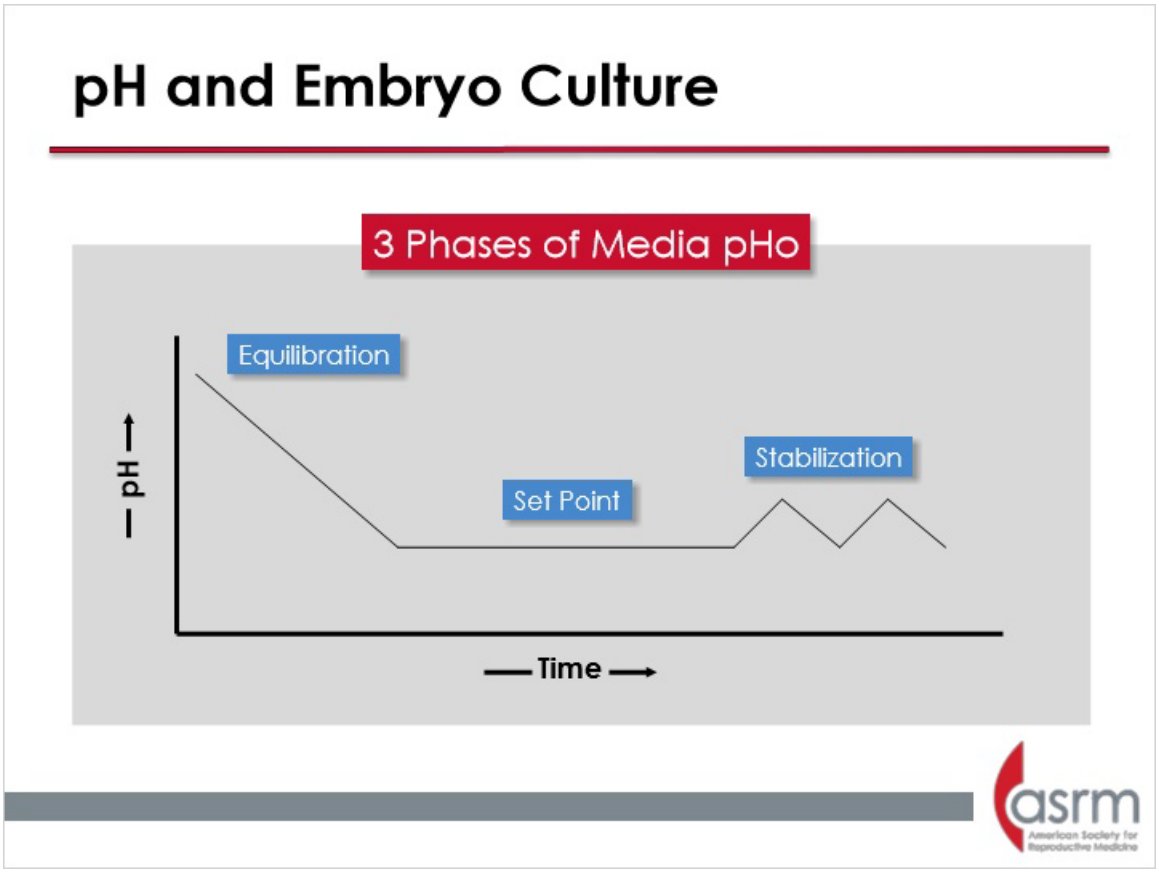
1.41 pH and ART



Notes:

What makes pHo a special topic of concern in IVF is that these cells are very sensitive to pH. Notably, denuded oocytes lack robust regulatory mechanisms, and procedures such as cryopreservation compromise the ability of embryos to regulate internal pHi. Recall what very minor changes in pHi for short periods of 3-4 hours can do in embryos and offspring. Additionally, sperm pHi and function can readily be influenced by pHo. Thus, maintenance of external pHo of the culture media is crucial to help maintain internal pHi and to avoid detrimental perturbations to cells.

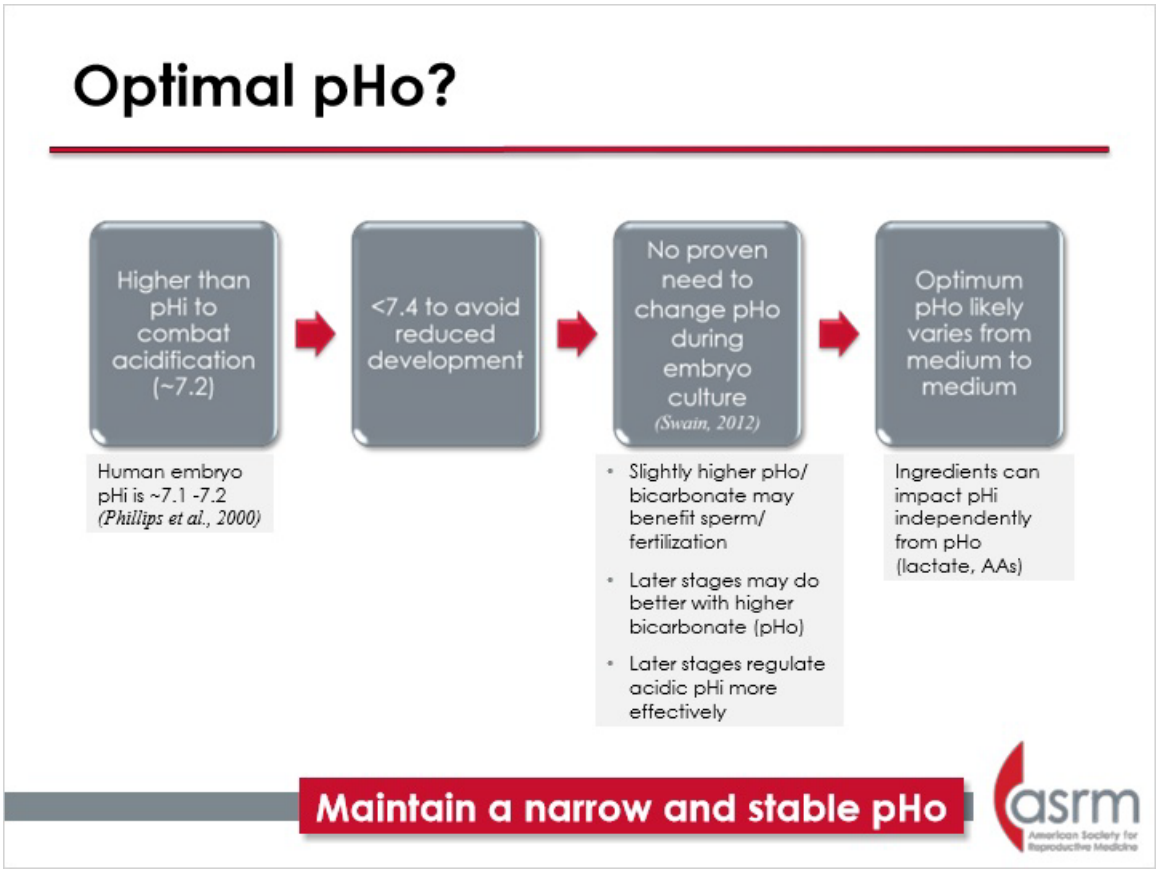
1.42 pH and Embryo Culture



Notes:

Referring back to media pHo, it is helpful to think of pH as occurring in 3 phases. There is the time needed to equilibrate media so that it can achieve its proper set point prior to placing gametes or embryos into the media. This entails placing dishes into the incubator at least several hours before use. The set point entails finding the right balance between the incubator gas environment and culture media. Finally, there is stabilization, or preventing harmful oscillations in pH during routine embryo handling. There are various methods to achieve pH stabilization, including minimizing incubator door openings, or use of other media components, such as pH buffers or mineral oil. Each of these will be discussed briefly.

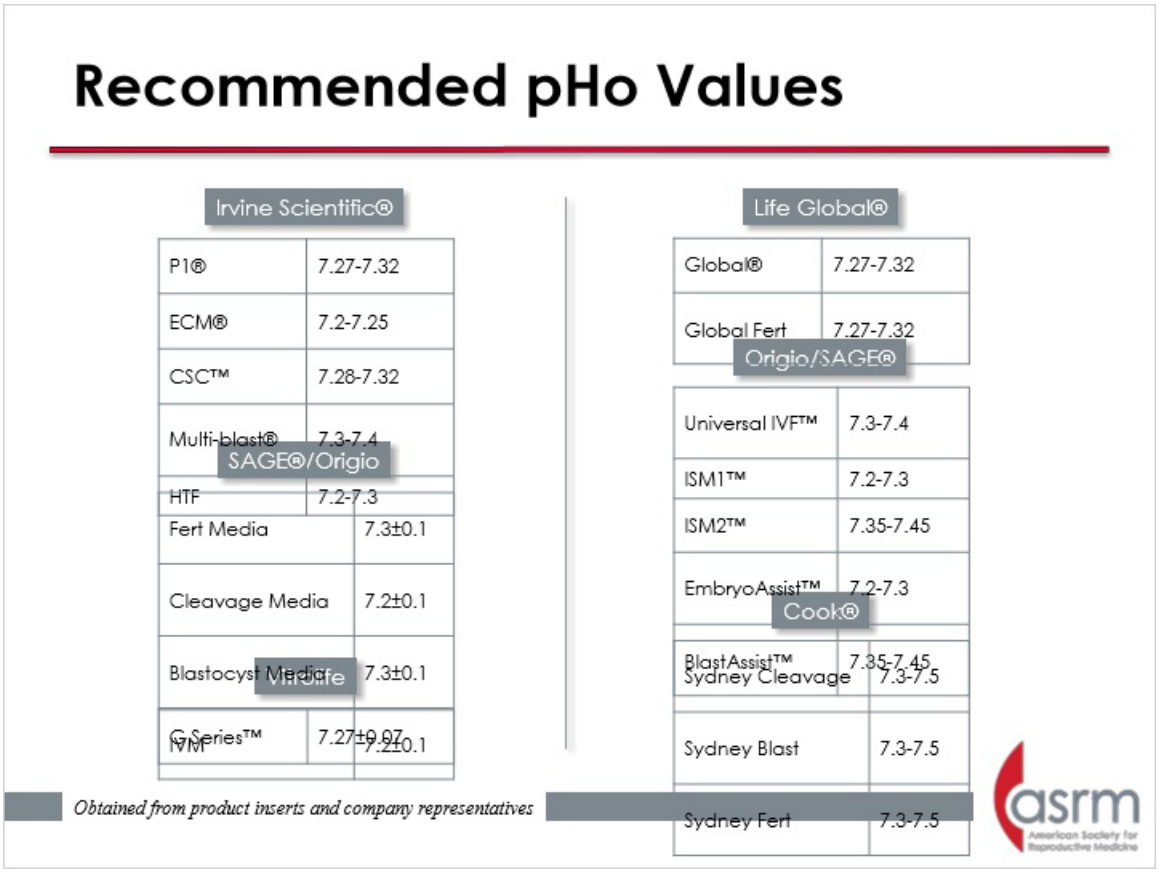
1.43 Optimal pHo?



Notes:

What should be the media pHo? Conventional wisdom is that the pHo should be slightly higher than pHi, which is ~7.1. So, the lower end of pHo is generally recommended to be 7.2. It should also be <7.4, which is where embryo development starts to decrease. There are no data to prove that a changing pH is required for embryo culture. A slightly higher pHo, or perhaps more likely bicarbonate concentration, may help benefit sperm and fertilization. Also, later stages of embryo development can regulate pHi more robustly than cleavage stages, so they may be able to tolerate a higher pHo, but this does not prove that it is required.

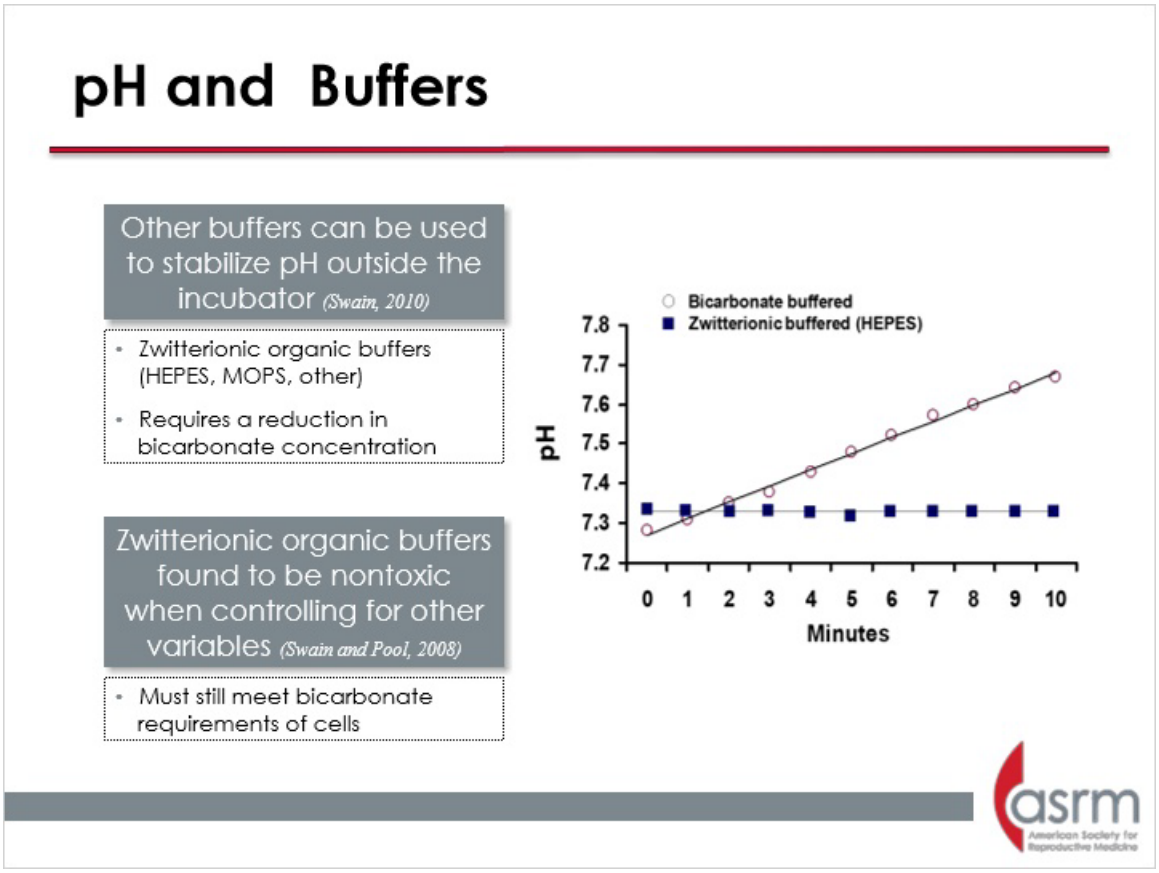
1.44 Recommended pHo Values



Notes:

Recommended pHo values for various commercial embryo culture media can be seen here. Most fall between 7.2 and 7.4, with some having tighter ranges than others.

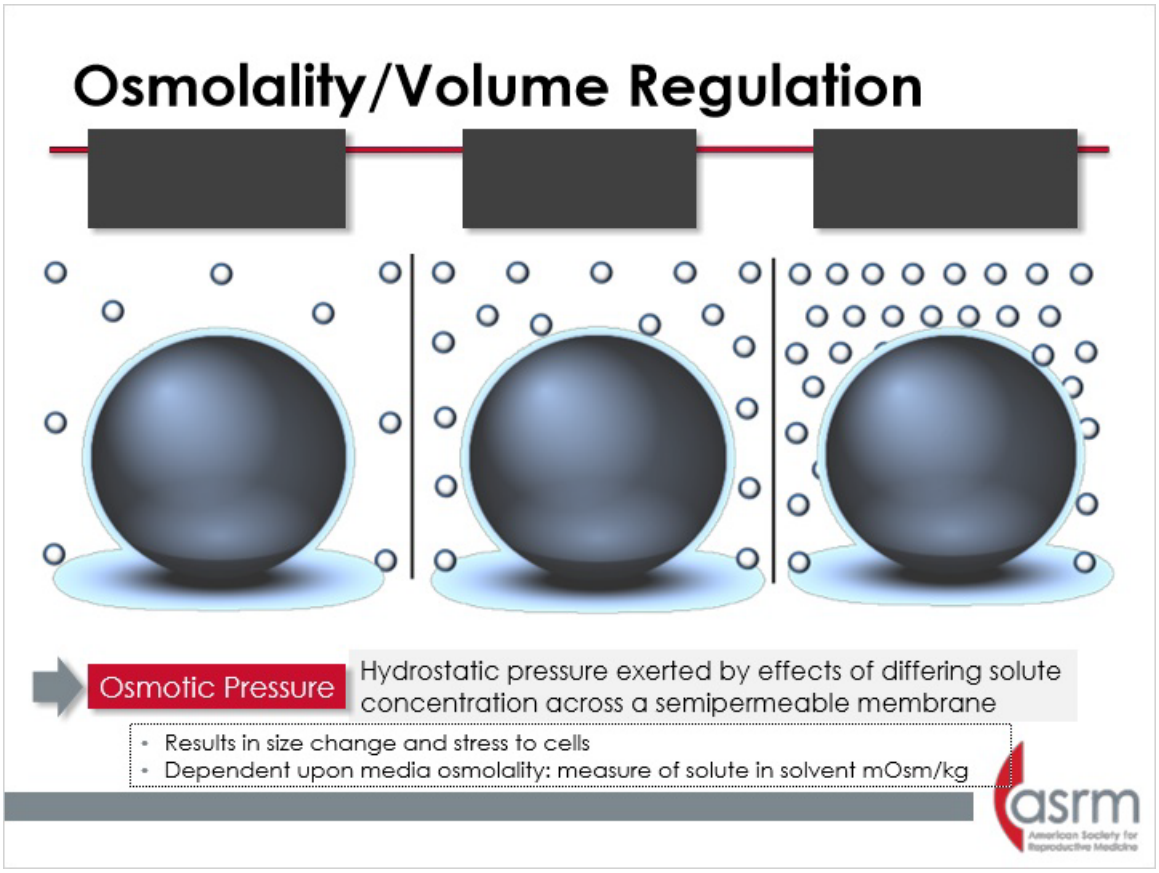
1.45 pH and Buffers



Notes:

Finally, other buffers besides bicarbonate can be used to stabilize media pH outside the confines of the laboratory incubator. Use of these buffers does not require elevated CO_2. As shown in the graph, when compared over 10 minutes using real-time pH measuring, use of a zwitterionic buffer, like HEPES, maintains a stable pH, while bicarbonate-buffered media quickly become alkaline when outside of the incubator. Buffers such as HEPES and MOPS are commonly used for procedures like retrieval, cryopreservation, ICSI, or transfer. Importantly, these media required a reduction in bicarbonate concentrations to stabilize pH. While some concern exists about potential embryo toxicity, it has been shown repeatedly that these buffers are safe when used appropriately and controlling for other confounding factors. Notably, cells do have a bicarbonate requirement, so this needs to be taken into account.

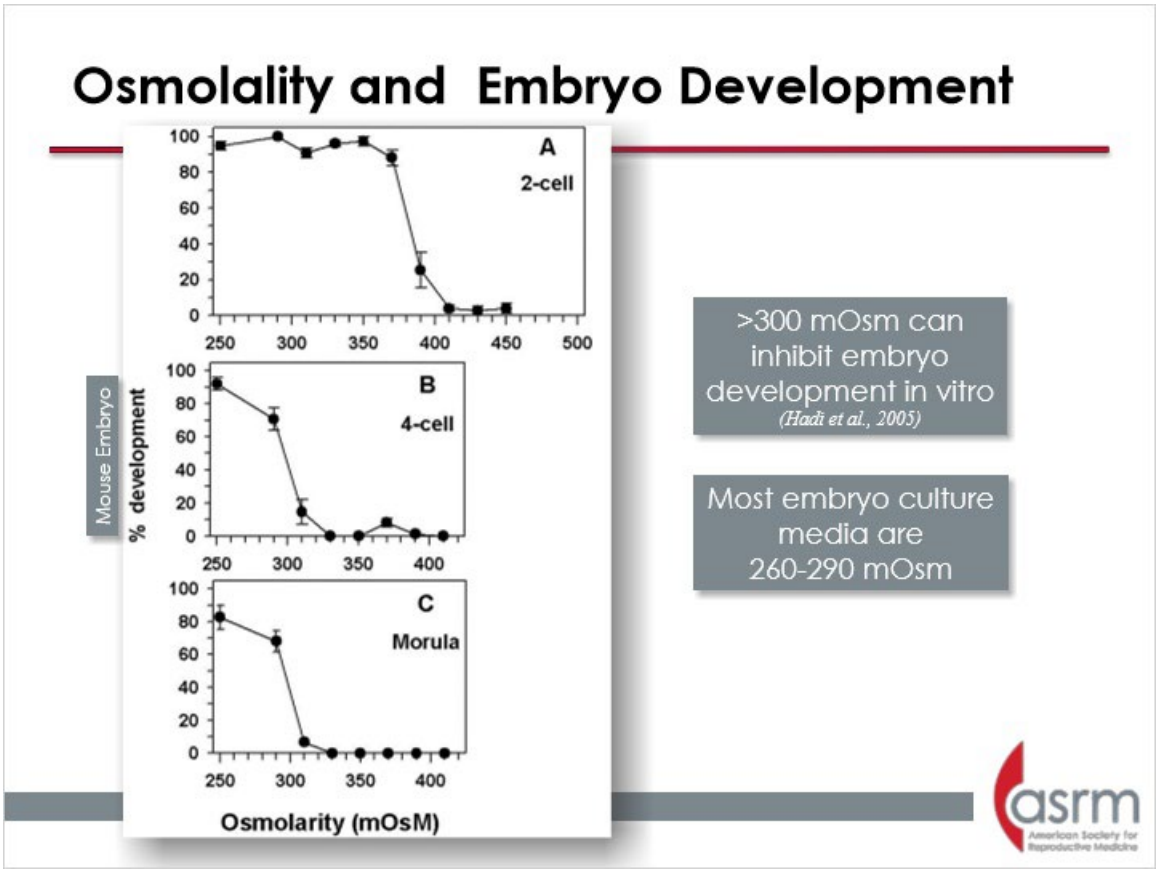
1.46 Osmolality/Volume Regulation



Notes:

One of the other critical areas or properties of culture media that can be controlled within the lab is osmolality. This is important because it regulates cell volume. As a brief refresher, osmotic pressure is hydrostatic pressure exerted by effects of differing solute concentrations across a membrane. This results in size or volume change of cells and can be a damaging stressor. This osmotic pressure is therefore dependent upon media osmolality. Recall that a hypotonic solution has low osmolality and results in water rushing into the cell, which then expands. If this happens too quickly, cells can lyse. This is what sometimes happens coming out of cryo solutions. An isotonic solution is where there is no net difference in solute concentrations inside or outside the cell, and there is no volume change. This is what is commonly desired in embryo culture media. Finally, a hypertonic solution has a very high solute concentration, like a cryopreservation medium, and water rushes out of the cell, which then shrinks.

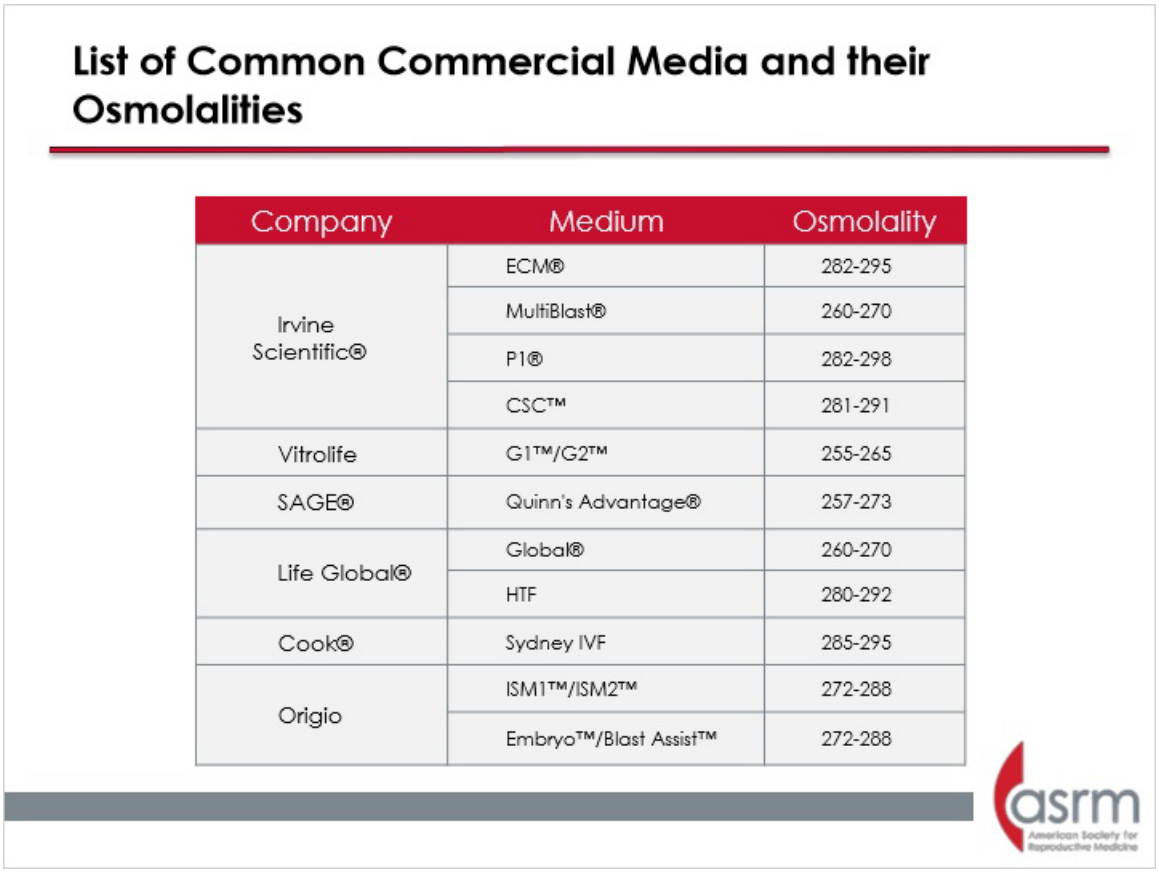
1.47 Osmolality and Embryo Development



Notes:

The reason this is important is that improper media osmolality can inhibit embryo development. This sensitivity will vary a bit depending on cell stage, but when media osmolality rises above around 300 mOsm, embryo development is compromised. This is why most embryo culture media are formulated to be between 260 and 290 mOsm.

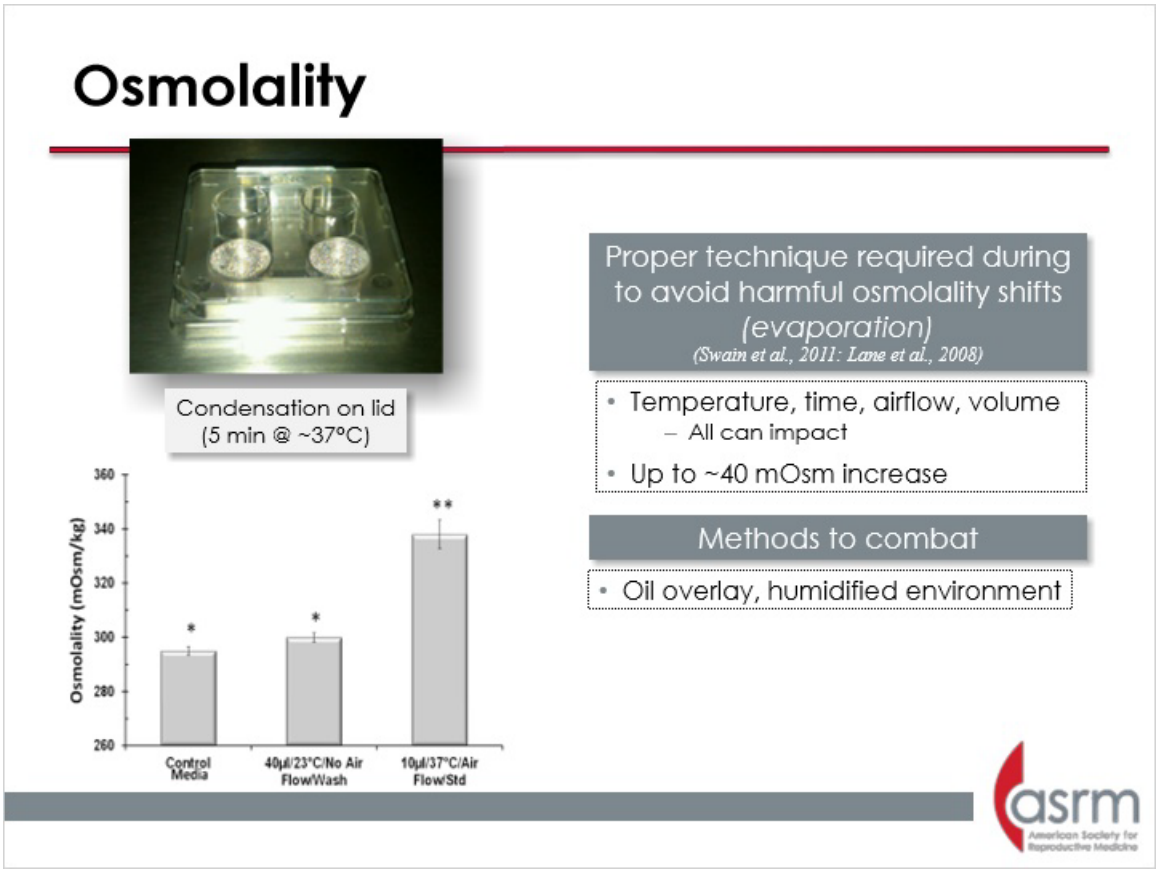
1.48 List of Common Commercial Media and their Osmolalities



Notes:

This is a list of common commercial media and their osmolalities. Note that they all fall between the 260-280 range.

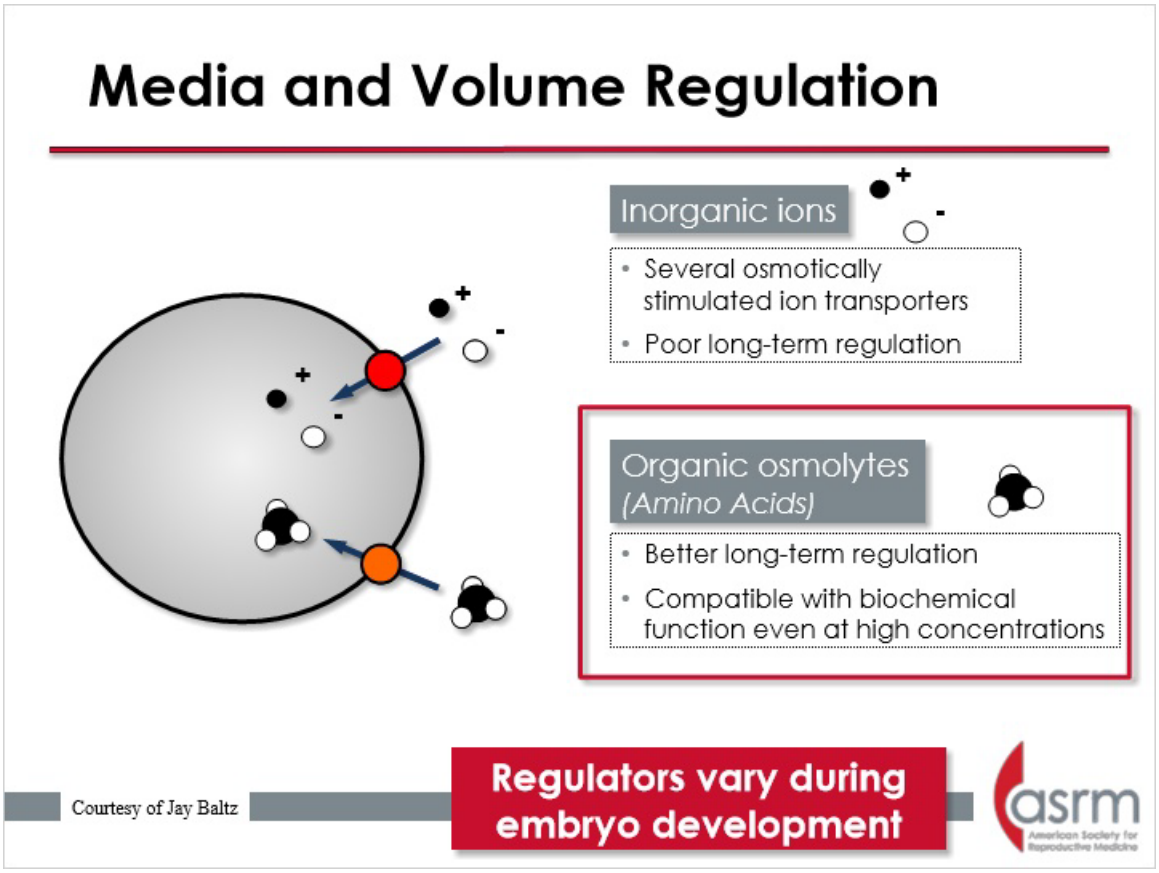
1.49 Osmolality



Notes:

Though companies set media osmolality in the correct range, improper techniques in the lab can inadvertently cause osmolality to rise to harmful levels. This is most commonly caused by media evaporation during storage or dish preparation or culture. Anytime condensation is seen, as shown here, this indicates evaporation and likely an osmolality shift. Factors that need to be considered to avoid this shift are temperature, time of preparation, airflow, and volume. One study showed that not adequately controlling for these factors during dish preparation resulted in a 40 mOsm increase, which was enough to inhibit embryo development. Employing methods to combat this shift include oil overlay and/or use of a humidified environment in the incubator.

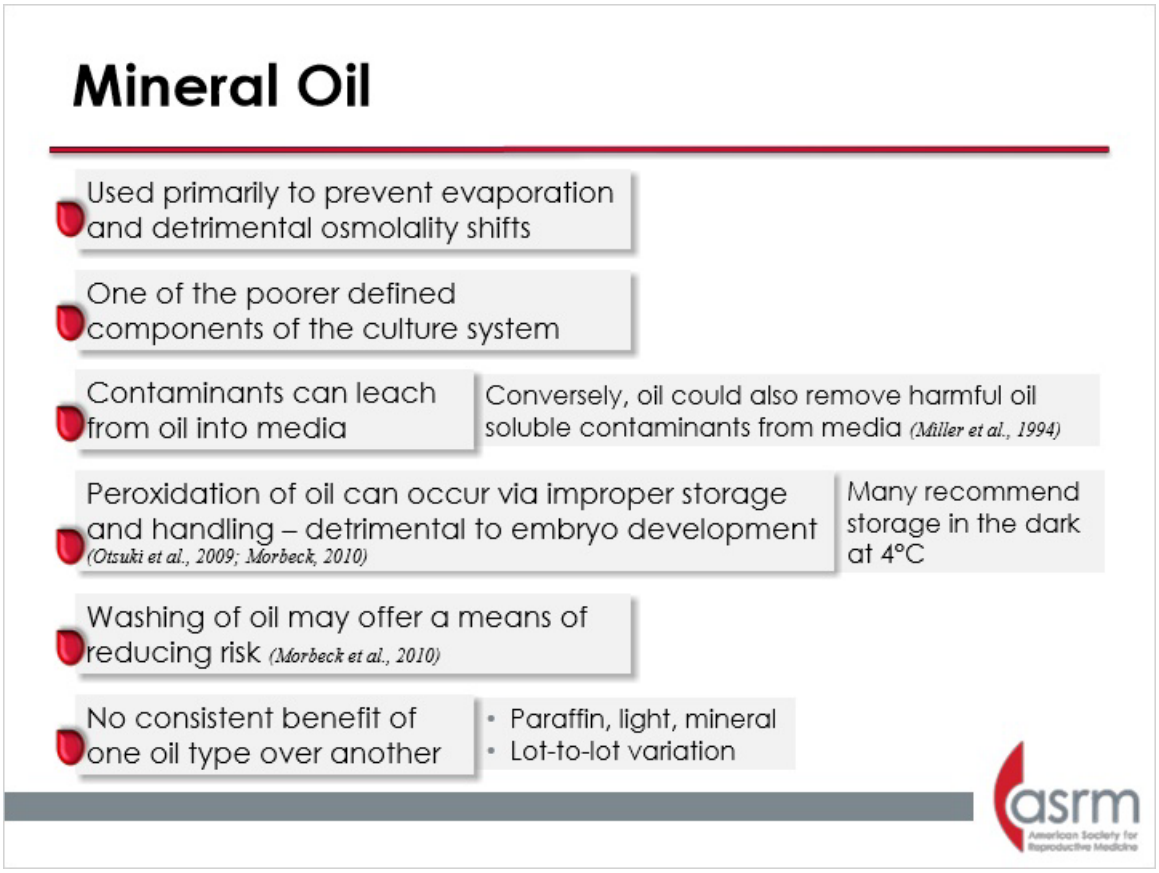
1.50 Media and Volume Regulation



Notes:

Remember that salts and ions play a role in culture media formulation and volume regulation. These are largely responsible for media osmolality and can themselves be used to help regulate cell volume via various transporters. However, this is a poor long-term solution. A better method of regulating cell volume is via organic osmolytes (amino acids). This is one of the primary reasons for their inclusion in media. Notably, regulation varies during embryo development, with different ions and osmolytes being functional at different developmental stages.

1.51 Mineral Oil

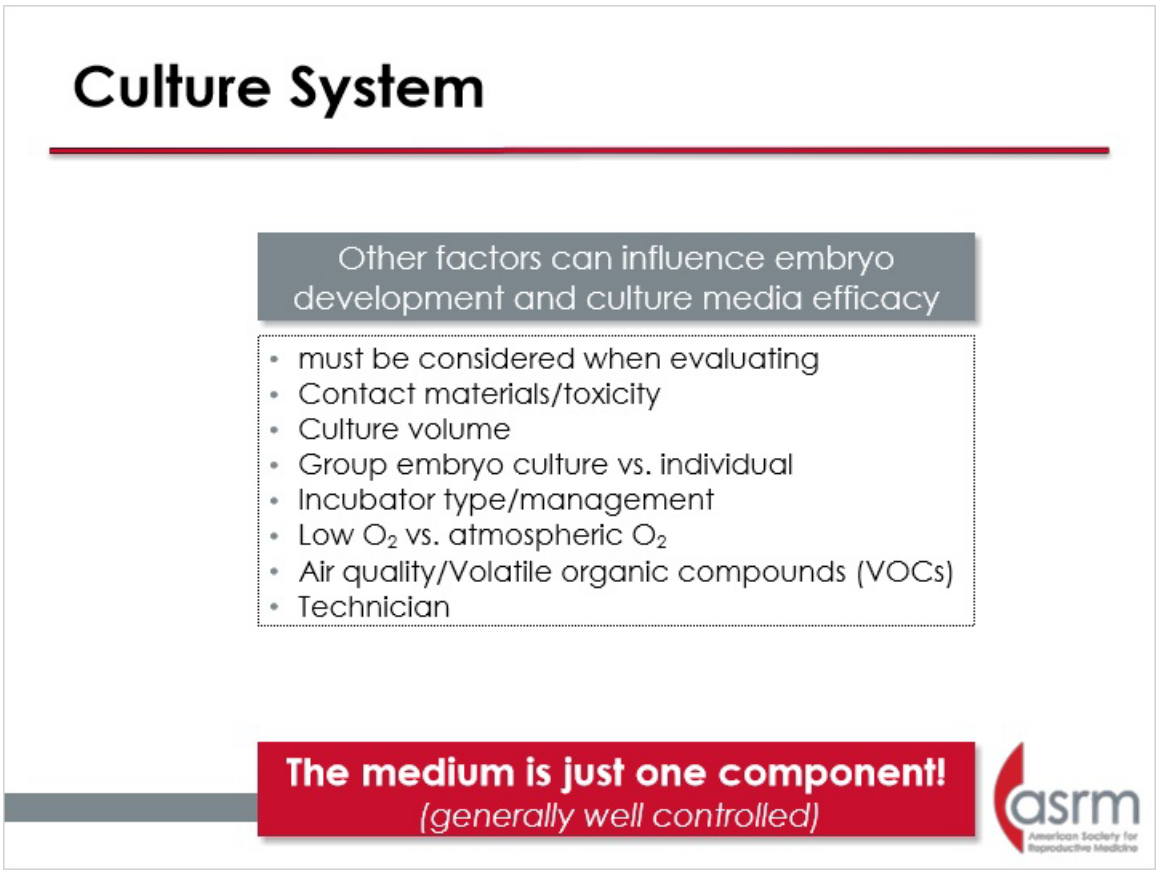


Notes:

The last area of culture media that will be discussed is mineral oil. While not a component of culture media per se, oil does directly contact and impact culture media. As mentioned, oil overlay is used to prevent evaporation and osmolality shifts of media. However, it is one of the poorer defined components of the culture system and has significant variability in its consistency. There are various reports in the literature about contamination or compromised embryo development due to bad mineral oil. Conversely, oil could help remove harmful, oil-soluble components from media, though the former scenario seems to be most often the case. One cause for mineral oil toxicity appears to be peroxidation that can be exacerbated by improper storage and handling of the oil. For this reason, many suppliers and labs now recommend protecting oil from light and storing it at lower temperatures. Some are even selling oil in glass bottles. Washing of oil has also been shown to help remove some of the toxic components. Prewashed oil can be purchased, or washing can occur inside the lab. Pure water and culture media both appear suitable for this process. Unfortunately, although there are now several products on the market, such as paraffin, light, and other types of oil, there does not seem to be a clear consensus on which is best. Realistically, these oils are mixtures, and there is lot-to-lot variation. Thus, it is likely up to rigorous quality control

by the supplier and/or lab to determine which product is most suitable for a particular laboratory.

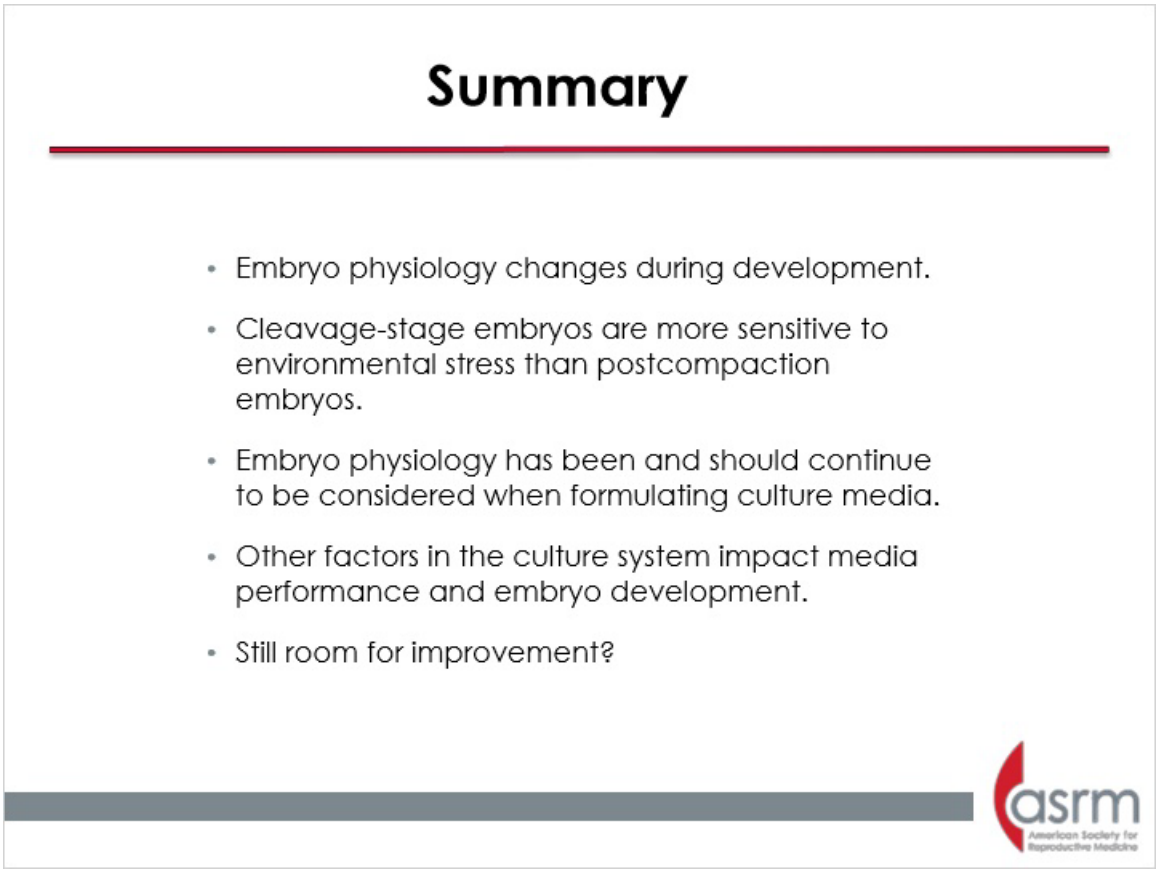
1.52 Culture System



Notes:

Finally, the culture medium is just one of the components of the culture system. This presentation began with a discussion of how many media can be used in a single lab, and then explored the various components of the embryo culture media. However, a number of other variables can impact efficacy of the culture media and must also be considered when evaluating the success of particular media. Remember, the medium is just one small component-perhaps one of the better controlled components-and other factors need to be considered to optimize in vitro embryo culture.

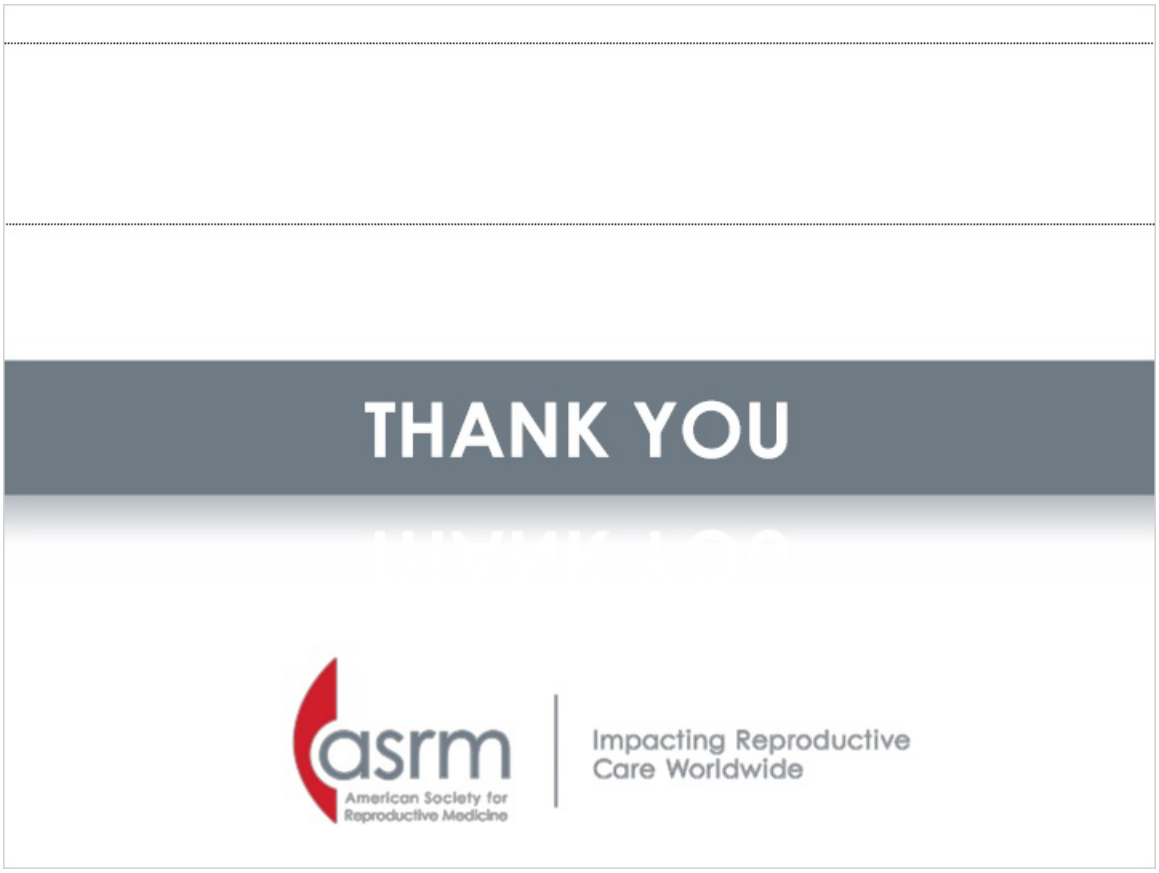
1.53 Summary



Notes:

In summary, embryo physiology changes during development, with cleavage-stage embryos being more sensitive to stressors than later stages of embryos. This physiology has helped shaped culture media formulations. However, there are other factors influencing culture media performance, other than just the medium. There remains room for improvement, whether it be growth factors or some other area of research.

1.54 Thank You



Notes:

Thank you for participating in this educational activity.