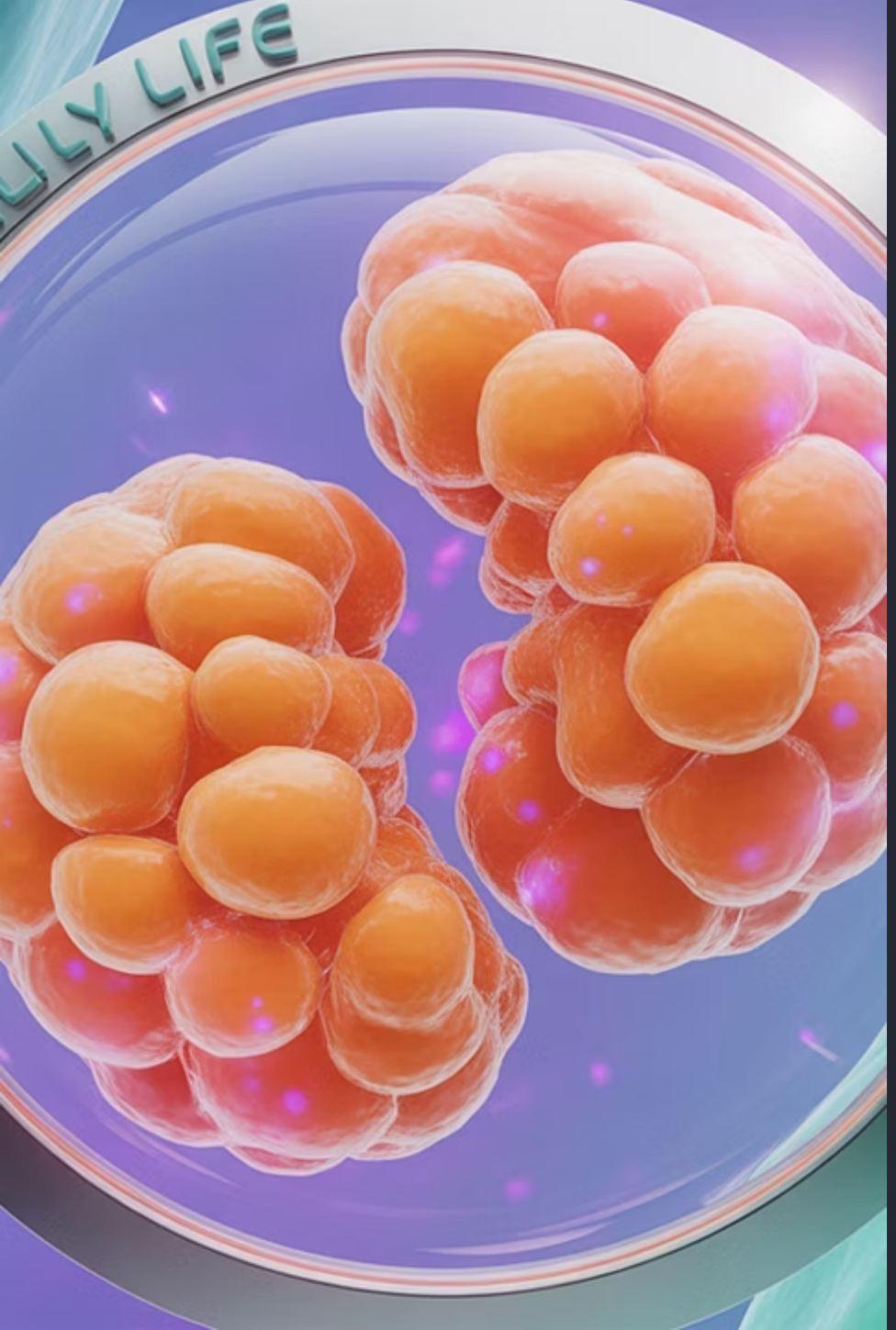


Cleavage-Stage Embryo Assessment and Grading

This course provides embryologists and IVF professionals with comprehensive training in the evaluation and grading of cleavage-stage human embryos. Accurate grading at this stage is essential for predicting developmental potential and selecting embryos with the highest likelihood of forming viable blastocysts and resulting in successful pregnancies. Participants will review morphological and molecular indicators of embryo quality, practice scoring embryos based on blastomere characteristics, and gain familiarity with developmental biology concepts that underpin cleavage-stage assessment.

by Fertility Guidance Technologies

Learning Objectives

1. Identify and describe the morphological features used in cleavage-stage embryo grading.
2. Evaluate the quality of embryos based on blastomere number, size, symmetry, and fragmentation.
3. Understand the biological mechanisms influencing embryo quality, including gene expression and signaling pathways.
4. Score embryo quality accurately using standardized criteria and image-based practice.
5. Explain how cleavage-stage grading impacts blastocyst development and clinical decision-making.

Interactive Assignment Instructions

1

Evaluate Embryo Images

Participants will evaluate a series of cleavage-stage embryo images.

2

Rate Embryo Quality

Rate the quality of each embryo based on visual cues.

3

Provide Written Descriptions

Provide brief written descriptions explaining your assigned grade.

4

Mobile Device Instructions

On mobile devices, use pinch and drag gestures to zoom or reposition images.

5

View Instructional Video

Tap the instructional video above to view a demonstration of grading techniques; full-screen mode is available via the expand icon.

Understanding Cleavage

After fertilization, the development of a multicellular organism proceeds by a process called **cleavage**, a series of mitotic divisions whereby the enormous volume of egg cytoplasm is divided into numerous smaller, nucleated cells. These cleavage-stage cells are called **blastomeres**.

In most species (mammals being the chief exception), the rate of cell division and the placement of the blastomeres with respect to one another is completely under the control of the proteins and mRNAs stored in the oocyte by the mother. The zygotic genome, transmitted by mitosis to all the new cells, does not function in early-cleavage embryos.

Few, if any, mRNAs are made until relatively late in cleavage, and the embryo can divide properly even

Characteristics of Cleavage

No Volume Increase

During cleavage, cytoplasmic volume does not increase. Rather, the enormous volume of zygote cytoplasm is divided into increasingly smaller cells. First the egg is divided in half, then quarters, then eighths, and so forth.

Rapid Cell Division

Division of egg cytoplasm without increasing its volume is accomplished by abolishing the growth period between cell divisions (that is, the G1 and G2 phases of the cell cycle). The cleavage of nuclei occurs at a rapid rate never seen again (not even in tumor cells).

Changing Nuclear-Cytoplasmic Ratio

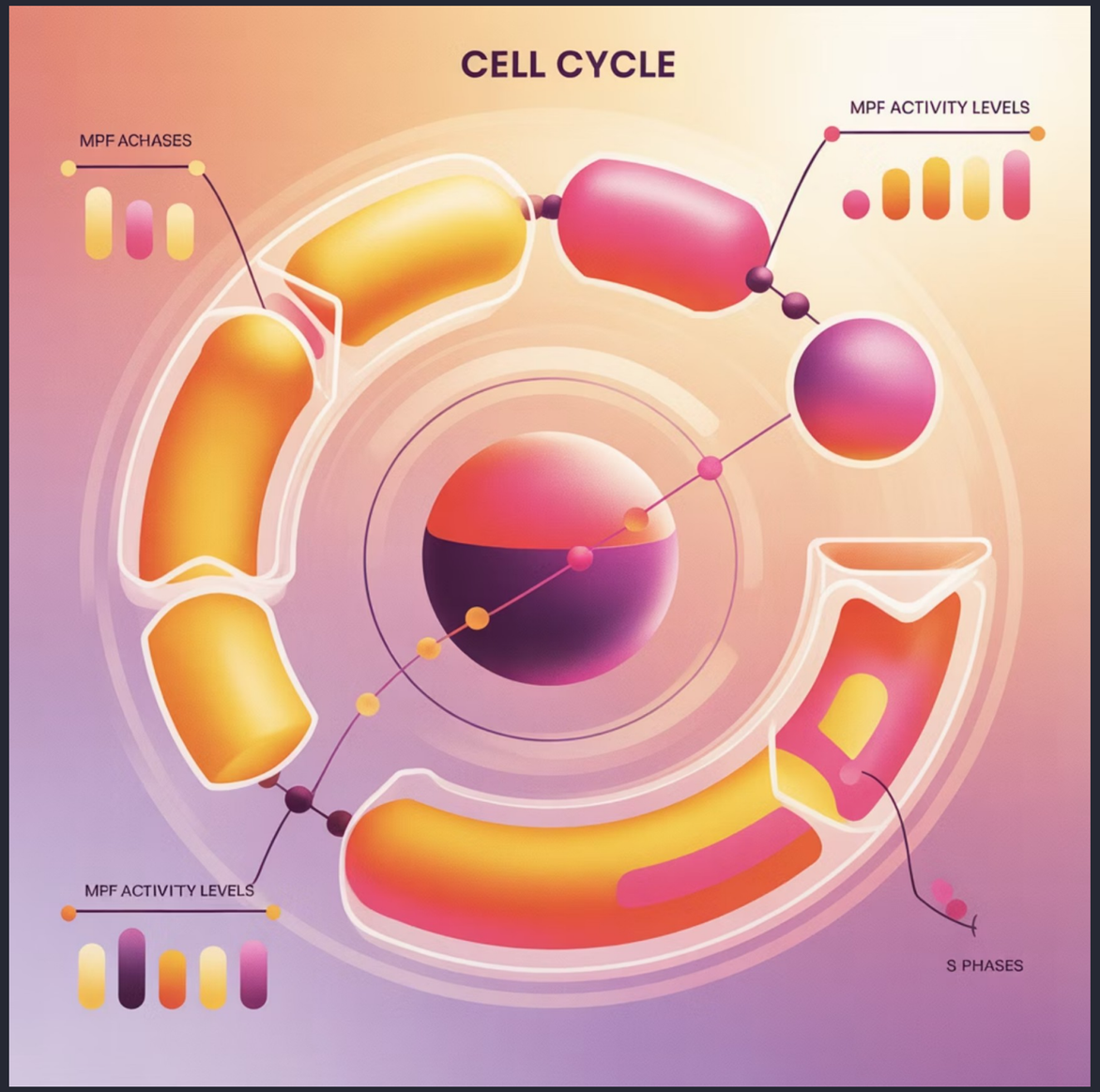
One consequence of this rapid cell division is that the ratio of cytoplasmic to nuclear volume gets increasingly smaller as cleavage progresses. In many types of embryos, this decrease in the cytoplasmic to nuclear volume ratio is crucial in timing the activation of certain genes.

For example, in the frog *Xenopus laevis*, transcription of new messages is not activated until after 12 divisions. At that time, the rate of cleavage decreases, the blastomeres become motile, and nuclear genes begin to be transcribed. This stage is called the **mid-blastula transition**.

From Fertilization to Cleavage

The transition from fertilization to cleavage is caused by the activation of **mitosis promoting factor** (**MPF**). MPF was first discovered as the major factor responsible for the resumption of meiotic cell divisions in the ovulated frog egg. It continues to play a role after fertilization, regulating the biphasic cell cycle of early blastomeres.

Blastomeres generally progress through a cell cycle consisting of just two steps: M (mitosis) and S (DNA synthesis). Gerhart and co-workers (1984) showed that MPF undergoes cyclical changes in its level of activity in mitotic cells. The MPF activity of early blastomeres is highest during M and undetectable during S.



Newport and Kirschner (1984) demonstrated that DNA replication (S) and mitosis (M) are driven solely by

Cyclic Activity of MPF

Cyclin B Synthesis

Mitosis-promoting factor contains two subunits. The large subunit is called **cyclin B**. It is this component that shows a periodic behavior, accumulating during S phase.

Cyclin Degradation

After the cells have reached M phase, cyclin B is degraded, allowing the cell to exit mitosis and return to S phase.

Kinase Activation

Cyclin B regulates the small subunit of MPF, the **cyclin-dependent kinase**. This kinase activates mitosis by phosphorylating several target proteins.

Mitosis Initiation

Phosphorylation brings about chromatin condensation, nuclear envelope depolymerization, and the organization of the mitotic spindle.

Without cyclin, the cyclin-dependent kinase will not function. The presence of cyclin is controlled by several proteins that ensure its periodic synthesis and degradation. In most species studied, the regulators of cyclin (and thus, of MPF) are stored in the egg cytoplasm.

Mid-Blastula Transition

As the cytoplasmic components are used up, the nucleus begins to synthesize them. The embryo now enters the mid-blastula transition, in which several new phenomena are added to the biphasic cell divisions of the embryo:

Addition of Growth Phases

First, the growth stages (G1 and G2) are added to the cell cycle, permitting the cells to grow. Before this time, the egg cytoplasm was being divided into smaller and smaller cells, but the total volume of the organism remained unchanged.

Loss of Synchronicity

Second, the synchronicity of cell division is lost, as different cells synthesize different regulators of MPF.

3

New mRNA Transcription

Third, new mRNAs are transcribed. Many of these messages encode proteins that will become necessary for gastrulation.

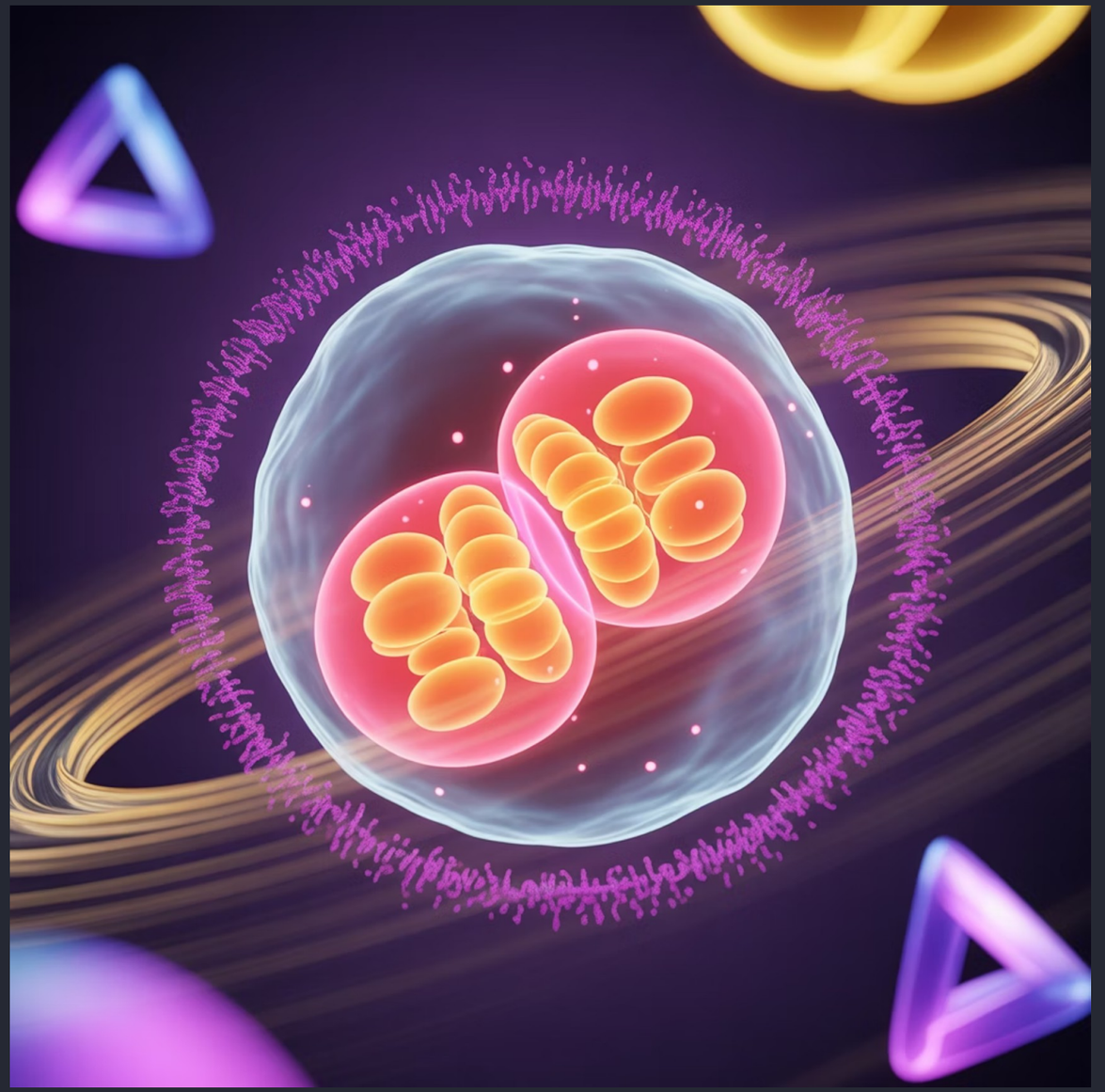
Xenopus embryos add those phases to the cell cycle shortly after the twelfth cleavage. *Drosophila* adds G2 during cycle 14 and G1 during cycle 17. If transcription is blocked, cell division will occur at normal rates and at normal times in many species, but the embryo will not be able to initiate gastrulation.

Cytoskeletal Mechanisms of Mitosis

Cleavage is actually the result of two coordinated processes:

Karyokinesis — the mitotic division of the nucleus. The mechanical agent of this division is the mitotic spindle, with its **microtubules** composed of **tubulin** (the same type of protein that makes up the sperm flagellum).

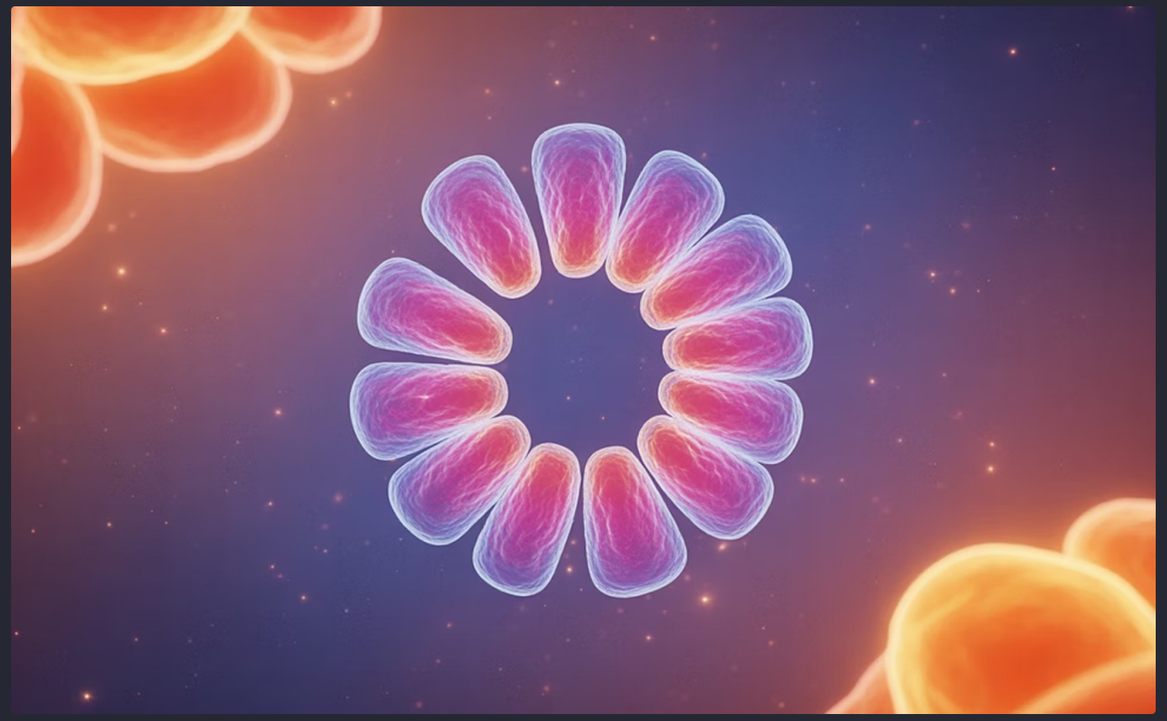
Cytokinesis — the division of the cell. The mechanical agent of cytokinesis is a **contractile ring** of **microfilaments** made of **actin** (the same type of protein that extends the egg microvilli and the sperm acrosomal process).

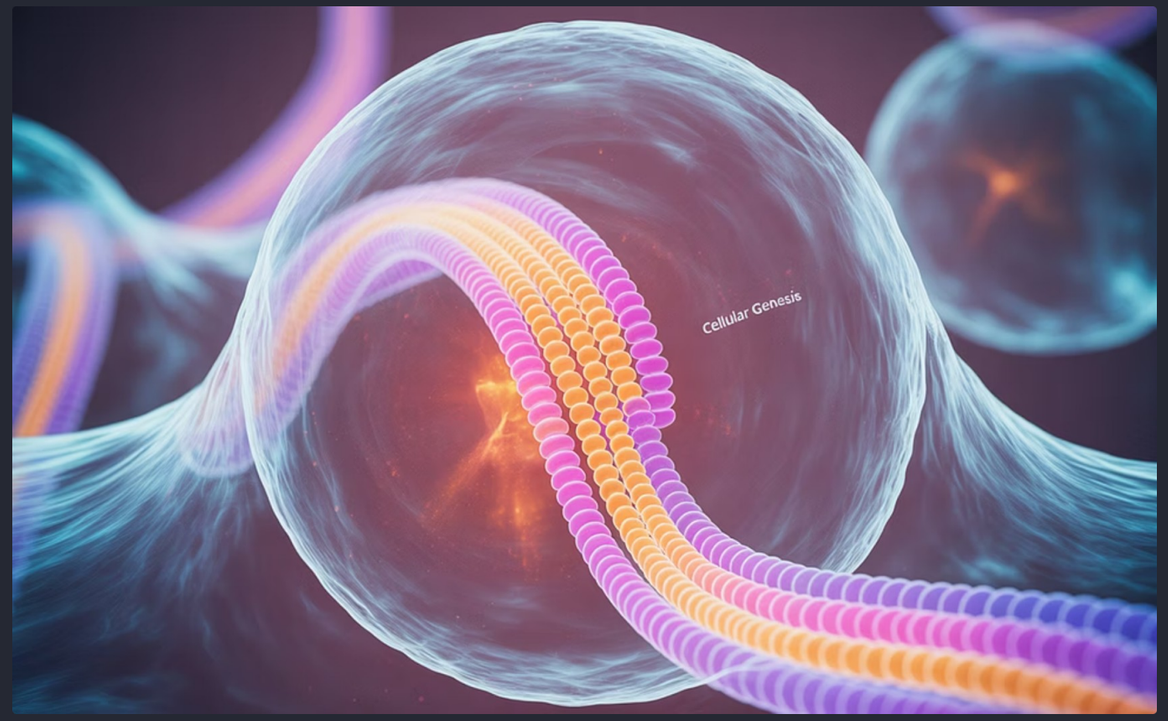


The mitotic spindle and contractile ring are perpendicular to each other, and the spindle is internal to the

Contractile Ring and Cleavage Furrow

The actin microfilaments are found in the cortex of the egg rather than in the central cytoplasm. Under the electron microscope, the ring of microfilaments can be seen forming a distinct cortical band 0.1 µm wide. This contractile ring exists only during cleavage and extends 8–10 µm into the center of the egg. It is responsible for exerting the force that splits the zygote into blastomeres; for if it is disrupted, cytokinesis stops.





Contractile Ring Structure

Schroeder (1973) has proposed a model of cleavage wherein the contractile ring splits the egg like an "intercellular purse-string," tightening about the egg as cleavage continues. This tightening of the microfilamentous ring creates the cleavage furrow.

Microtubule Function

Microtubules are also seen near the cleavage furrow (in addition to their role in creating the mitotic spindles), since they are needed to bring membrane material to the site of membrane addition.

Coordination of Karyokinesis and Cytokinesis

Although karyokinesis and cytokinesis are usually coordinated, they are sometimes separated by natural or experimental conditions. In insect eggs, karyokinesis occurs several times before cytokinesis takes place.

Another way to produce this state is to treat embryos with the drug cytochalasin B. This drug inhibits the formation and organization of microfilaments in the contractile ring, thereby stopping cleavage without stopping karyokinesis.

Process	Mechanical Agent	Protein Component
Karyokinesis	Mitotic spindle	Tubulin (microtubules)
Cytokinesis	Contractile ring	Actin (microfilaments)

When these processes are experimentally separated, we can observe nuclear division without cell division, resulting in multinucleated cells.

Patterns of Embryonic Cleavage

In 1923, embryologist E. B. Wilson reflected on how little we knew about cleavage: "To our limited intelligence, it would seem a simple task to divide a nucleus into equal parts. The cell, manifestly, entertains a very different opinion." Indeed, different organisms undergo cleavage in distinctly different ways.

The pattern of embryonic cleavage particular to a species is determined by two major parameters:

1. The amount and distribution of yolk protein within the cytoplasm
2. Factors in the egg cytoplasm that influence the angle of the mitotic spindle and the timing of its formation

The amount and distribution of yolk determines where cleavage can occur and the relative size of the blastomeres. When one pole of the egg is relatively yolk-free, the cellular divisions occur there at a faster rate than at the opposite pole. The yolk-rich pole is referred to as the **vegetal pole**; the yolk concentration in the **animal pole** is relatively low. The zygote nucleus is frequently displaced toward the animal pole. In general, yolk inhibits cleavage.

Classification of Cleavage Types

Isolecithal Eggs (Sparse, Equally Spaced Yolk)

Found in sea urchins, mammals, and snails. In these species, cleavage is holoblastic (Greek holos, "complete"), meaning that the cleavage furrow extends through the entire egg. These embryos must have some other way of obtaining food. Most will generate a voracious larval form, while mammals get their nutrition from the placenta.

Telolecithal Eggs (Large Yolk Concentration)

Found in insects, fishes, reptiles, and birds. Most of their cell volumes are made up of yolk. Zygotes containing large accumulations of yolk undergo meroblastic cleavage, wherein only a portion of the cytoplasm is cleaved. The cleavage furrow does not penetrate into the yolky portion of the cytoplasm.

Holoblastic Cleavage Patterns

In the absence of a large concentration of yolk, four major cleavage types can be observed:

Radial Holoblastic

Cleavage planes are either parallel or perpendicular to the animal-vegetal axis of the egg. Found in echinoderms and amphibians.

Spiral Holoblastic

Cleavage planes are at oblique angles to the animal-vegetal axis, creating a spiral arrangement of blastomeres. Found in mollusks and annelids.

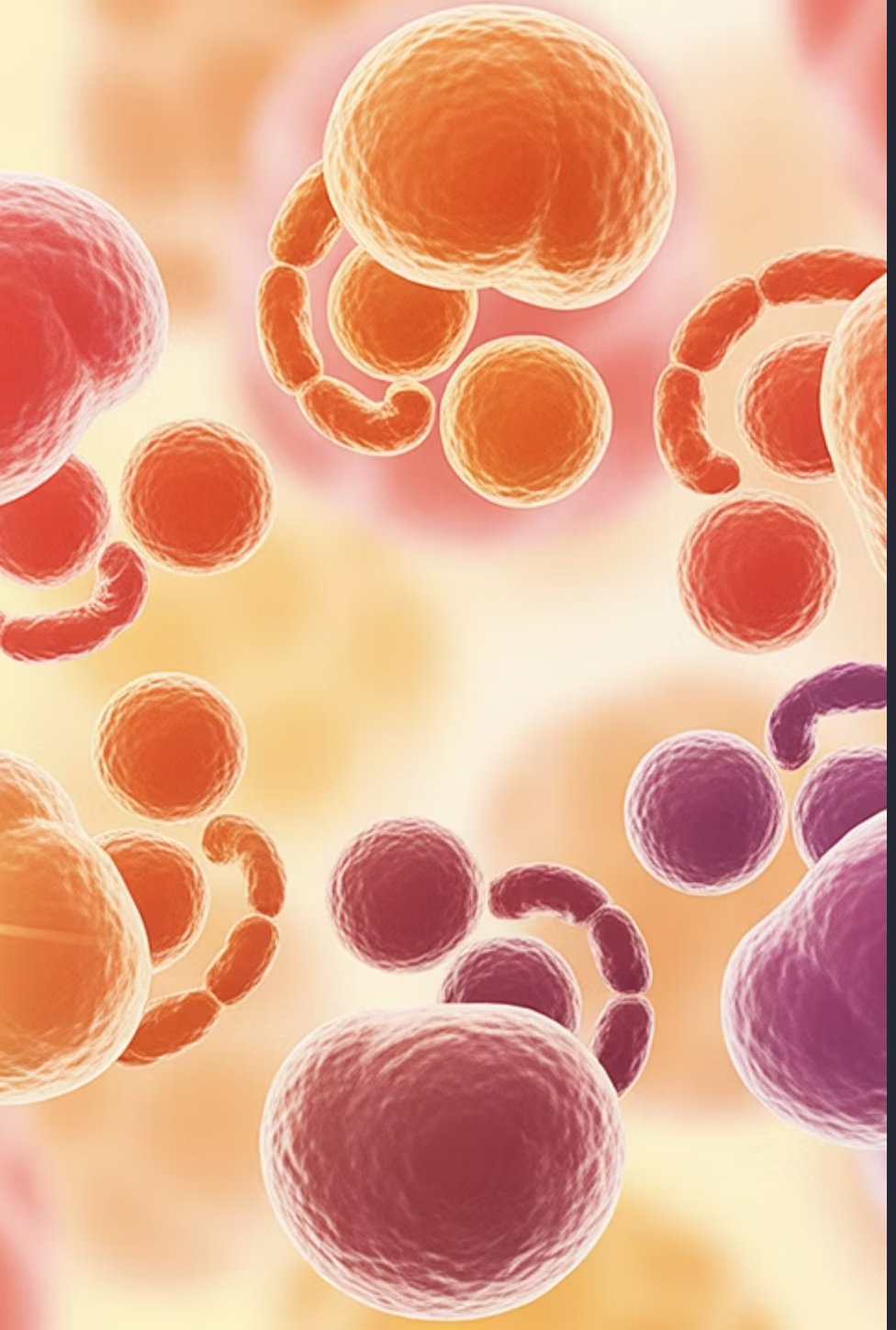
Bilateral Holoblastic

Cleavage results in blastomeres arranged with bilateral symmetry. Found in tunicates and cephalochordates.

Rotational Holoblastic

Unique pattern where the second cleavage plane is perpendicular to the first but only in one blastomere. Found in mammals.

These patterns are superimposed upon the constraints of the yolk and represent inherited patterns of cell division specific to different evolutionary lineages.



Embryo Quality Assessment Criteria

Blastomere Number

Optimal embryos typically have 4 cells at Day 2 and 8 cells at Day 3.

Too few or too many blastomeres may reflect chromosomal abnormalities or abnormal cleavage.

Blastomere Size Uniformity

Uniform blastomere size suggests synchronous cell divisions and healthy progression.

Size disparities can indicate asymmetric division or developmental delay.

Fragmentation

Fragmentation refers to cytoplasmic debris not enclosed in membranes.

High fragmentation (>25%) correlates with lower implantation potential.

Causes include abnormal cytokinesis or cellular stress.

Additional Quality Assessment Criteria

Blastomere Symmetry

Symmetry indicates even distribution of cytoplasm and cellular material.

Asymmetry is often associated with impaired developmental potential.

Developmental Rate

Development must occur within expected timeframes.

Accelerated or delayed cleavage can suggest aneuploidy or metabolic stress.

Cell-to-Cell Communication

Gap junctions allow metabolic and signaling coordination between blastomeres.

Disruption can impair further development and affect blastocyst formation.

Gene Expression and Regulation

During cleavage, dynamic gene expression is vital for pluripotency and differentiation.

Dysregulated transcription may impair embryo competence.

Molecular and Structural Biology of Cleavage-Stage Embryos

Par Complex

Regulates cell polarity and asymmetric division.

Ensures correct distribution of developmental signals during cleavage.

Hippo Signaling Pathway

Controls cell proliferation and fate through interactions with the cytoskeleton.

Crucial for early decisions between inner cell mass and trophectoderm lineages.

Wnt Signaling Pathway

Regulates blastomere polarity, proliferation, and spatial organization.

Abnormal Wnt signaling may impair compaction and later lineage allocation.

Mitotic Spindle Orientation

Correct alignment ensures even division and chromosomal segregation.

Misorientation can lead to blastomere asymmetry or aneuploidy.

Cytokinesis: Actin, myosin, and Rho GTPases drive final cell separation. Errors in this process may produce fragments or uneven cell sizes.

Grading Summary Table

Grading Criterion	High Quality Embryo	Moderate Quality Embryo	Poor Quality Embryo
Blastomere Number	4–8 (stage appropriate)	Slightly fewer/more	Significantly off-stage
Blastomere Size	Uniform	Mildly uneven	Markedly unequal
Fragmentation	<10%	10–25%	>25%
Symmetry	High symmetry	Minor asymmetry	Severe asymmetry
Cleavage Timing	On schedule	Slightly early/late	Delayed or abnormal rate

Practical Embryo Assessment

Visual Assessment Techniques

When evaluating cleavage-stage embryos, it's important to:

- Use standardized scoring systems
- Assess embryos at consistent time points (Day 2 and Day 3)
- Document all observations systematically
- Consider the embryo's developmental trajectory, not just a single timepoint

Time-lapse imaging systems can provide additional information about cleavage patterns and timing that may not be apparent in static observations.

Common Challenges

Clinical Applications and Future Directions

Traditional Morphological Assessment

Standard practice relies on visual evaluation of blastomere number, size, symmetry, and fragmentation to select embryos for transfer.

1

Time-Lapse Monitoring

Advanced imaging allows continuous observation of embryo development, revealing cleavage patterns and timing that correlate with implantation potential.

2

Non-Invasive Metabolomics

Analysis of culture media to assess embryo metabolism and secreted biomarkers as indicators of embryo health.

3

AI-Assisted Grading

Machine learning algorithms trained on thousands of embryo images can help standardize assessment and potentially identify subtle features correlated with success.

4

While new technologies continue to emerge, understanding the fundamental biology of cleavage-stage embryos remains essential for accurate assessment and selection of embryos with the highest developmental potential.