

Molecular and Cellular Biology (MCB)

BB101

**LECTURE-3
11/ 1/ 2018**

Development and Cell Reprogramming

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Outline

1. Animal Development
2. Cell Reprogramming and Cloning

A close-up, low-light microscopic image showing the early stages of embryonic development. Several large, pale blue cells are visible, with some internal structures and small red spots becoming discernible.

Development

How Did a Single Cell Develop Into Embryo?

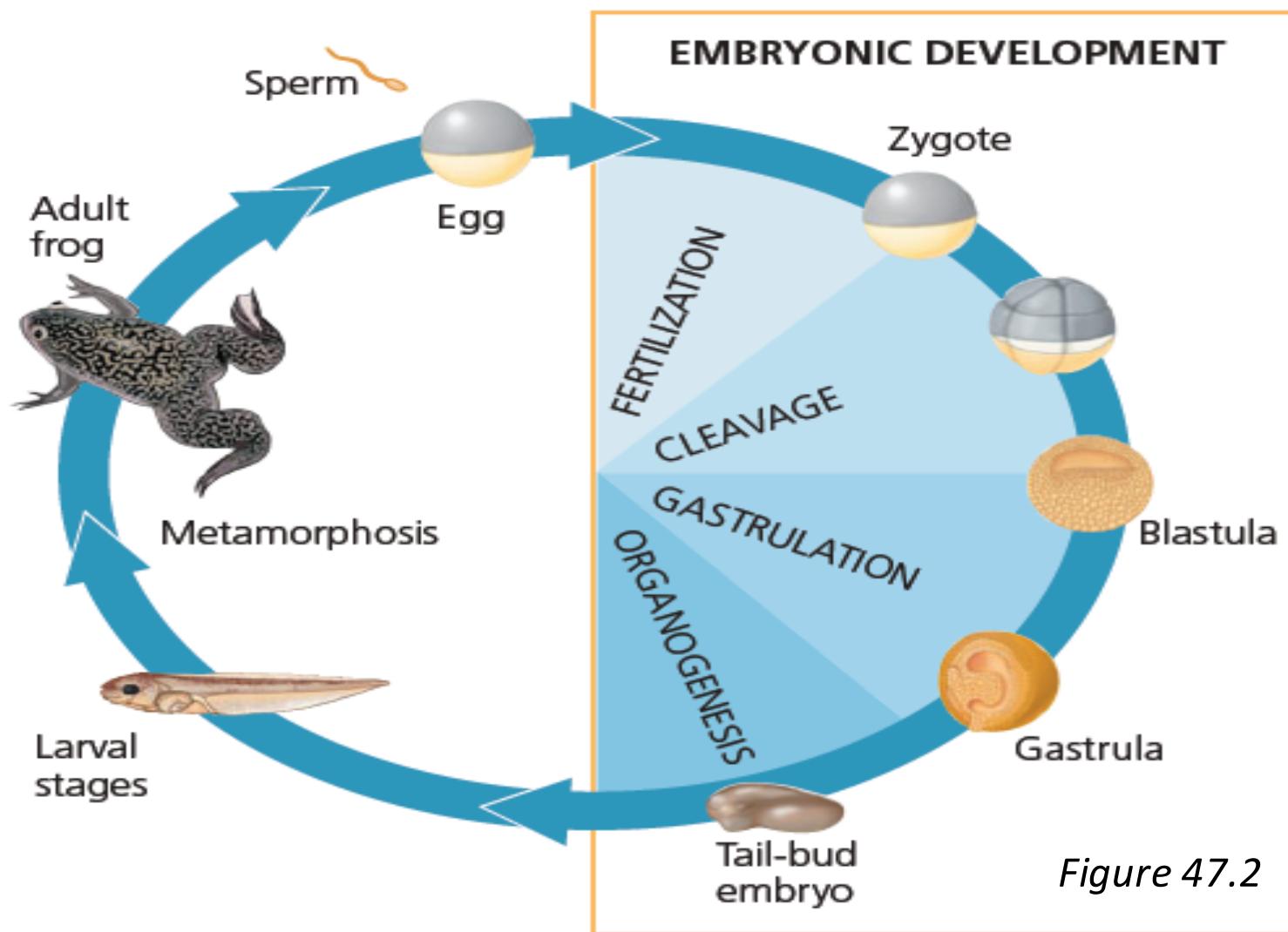
Why Should We Study Development?

- One of the most important processes in the human body
- Helped to reveal evolutionary connections
- Diseases/ misregulated development:
 - *cleft lip, heart disease, club foot, neural defects*

Common Stages of Embryonic Development

- **Fertilization** – fusion of sperm & egg
- **Cleavage** – series of cell divisions divide embryo into many cells & generate a hollow ball of cells “blastula”
- **Gastrulation** – Blastula rearranges into a multilayered embryo
- **Organogenesis** – last major stage of embryo development, which generates rudimentary organs

Common Stages of Embryonic Development



Metamorphosis - larva (tadpole) undergoes major changes in anatomy to become an adult

Fertilization in Mammals

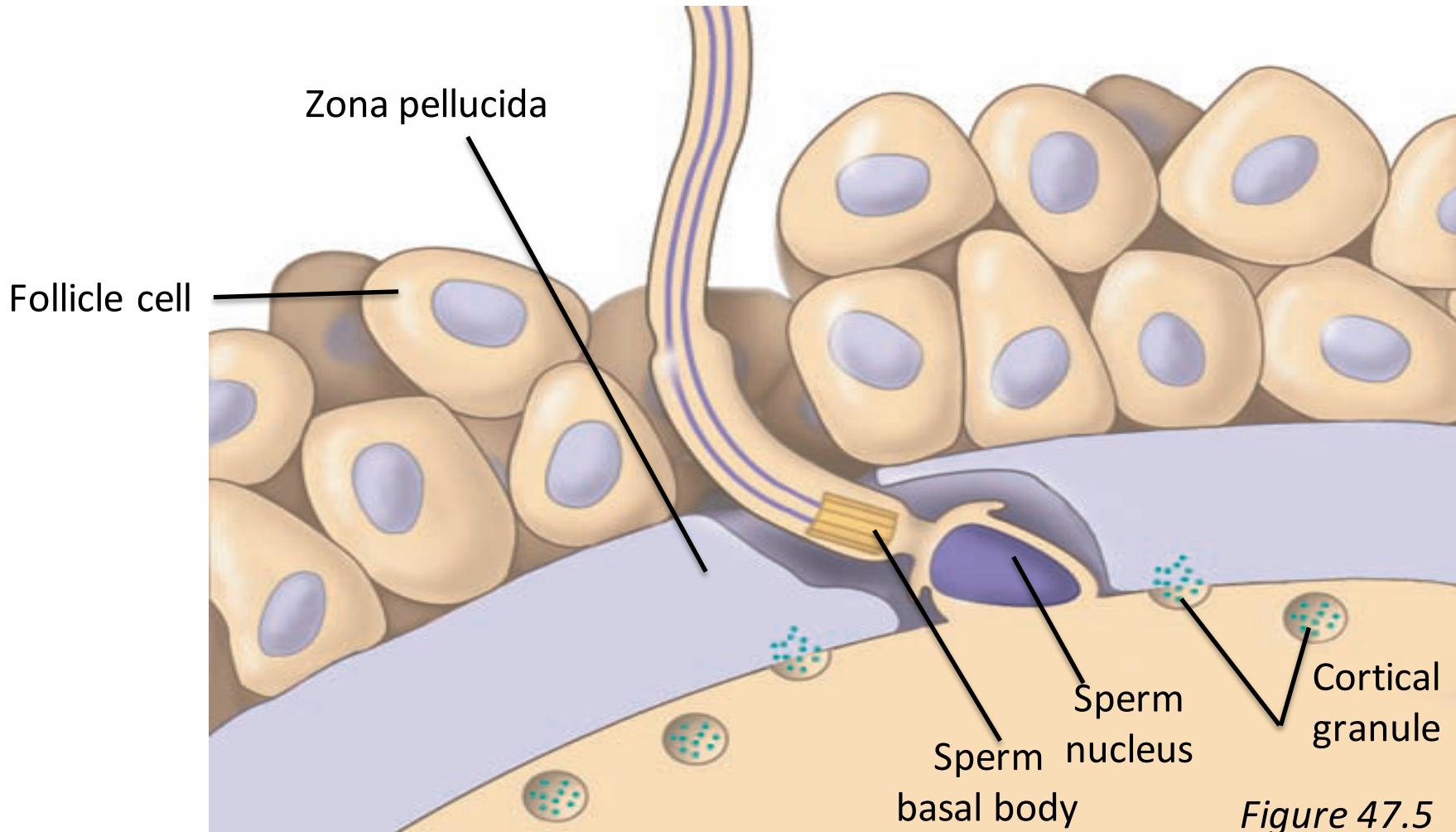
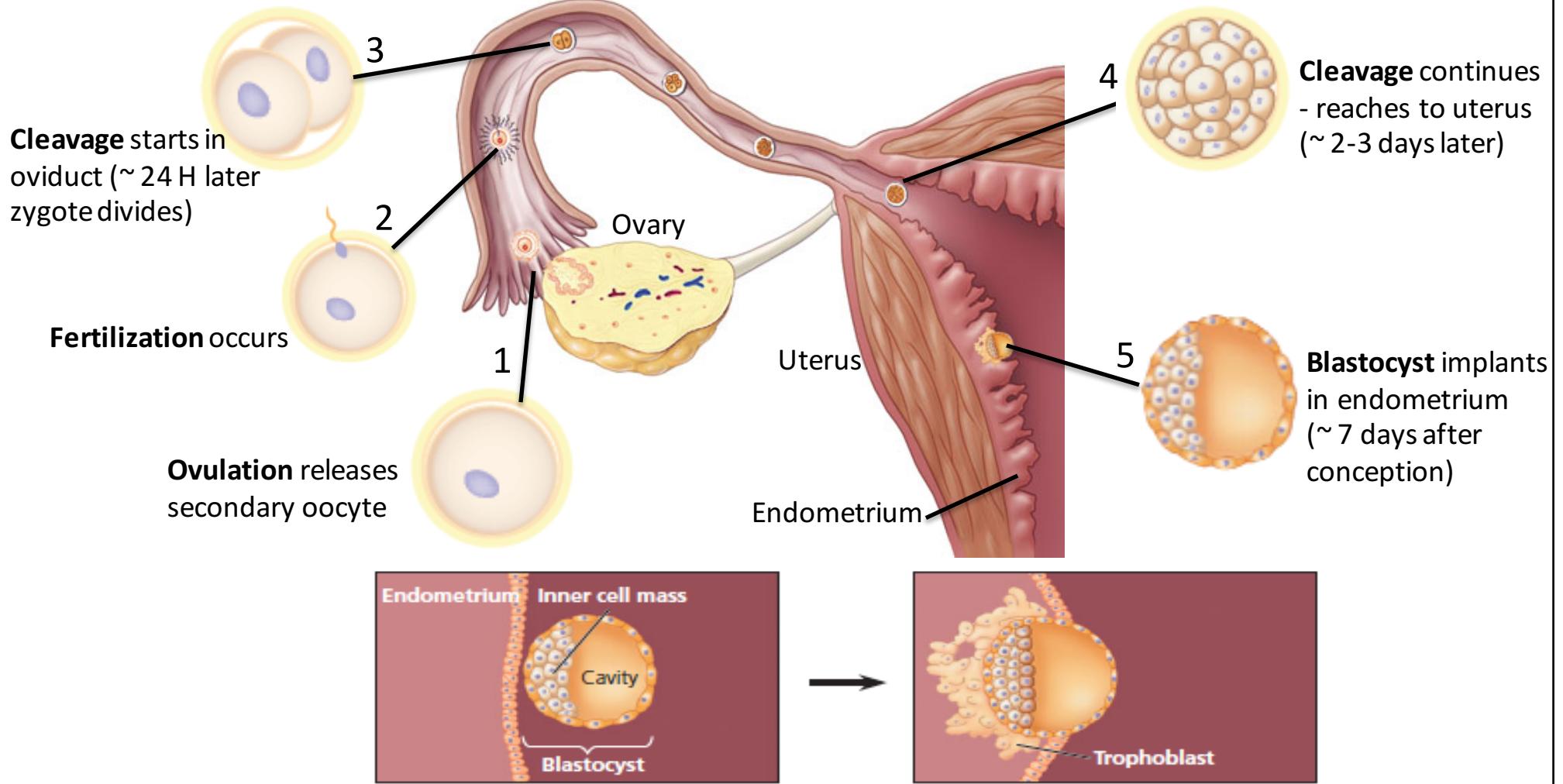


Figure 47.5

- Sperm travels through follicle cells & zona pellucida, & fuse with egg
- Cortical reaction initiates events to ensure that only one sperm nucleus enters the egg

Zygote formation & early Post-fertilization Events

From Ovulation to Implantation



Implantation of Blastocyst

Figure 46.15

Several days after blastocyst formation, embryo implants into the endometrium; Human pregnancy averages 38 weeks

Stages of Human Embryo Development

- Placenta is combination of maternal & embryonic tissues
- It transports nutrients, respiratory gases & wastes between embryo or fetus & mother

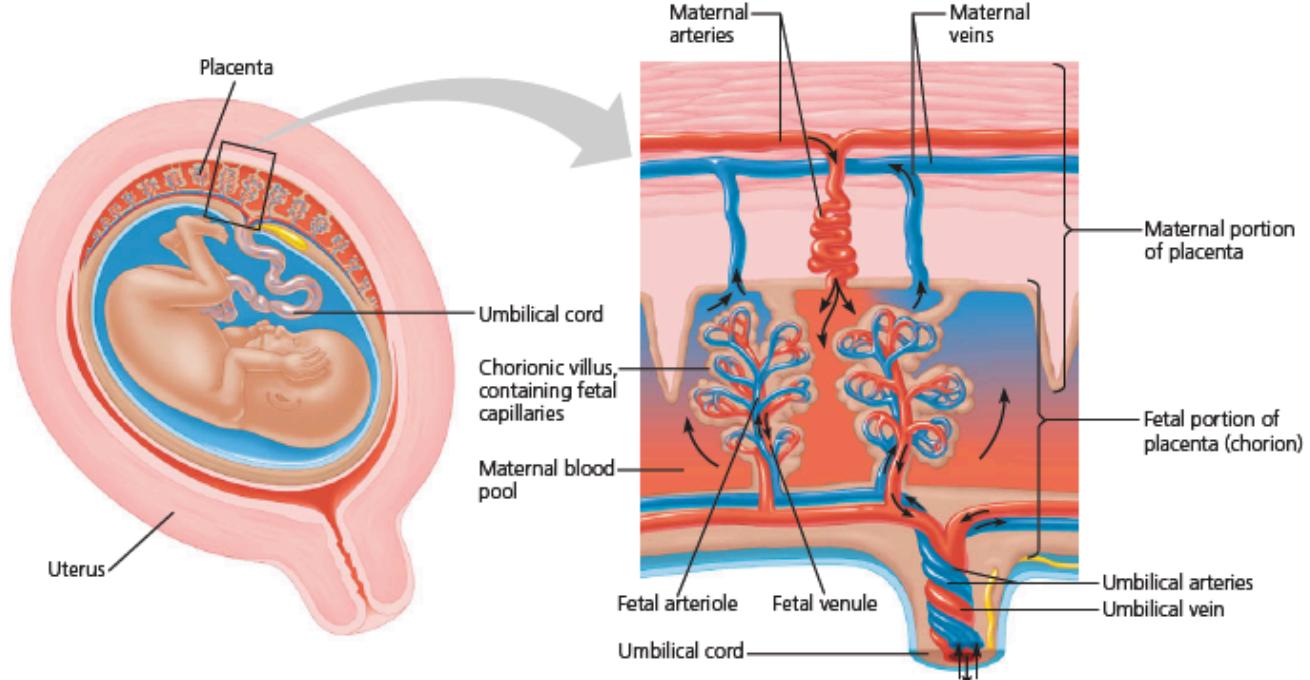


Figure 46.16



Shaping the Vertebrate Body

Germ layers: Cell layers produced by gastrulation

Ectoderm (Outer layer of embryo)

Epidermis of skin

Nervous and sensory system

Jaws and teeth

Germ cells

Mesoderm (middle layer of embryo)

Skeletal and muscular system

Dermis of skin

Excretory and reproductive systems

Circulatory and lymphatic system

Endoderm (Inner layer of embryo)

Epithelial lining of digestive tract,
respiratory tract and reproductive tract

Thymus, thyroid and parathyroid glands

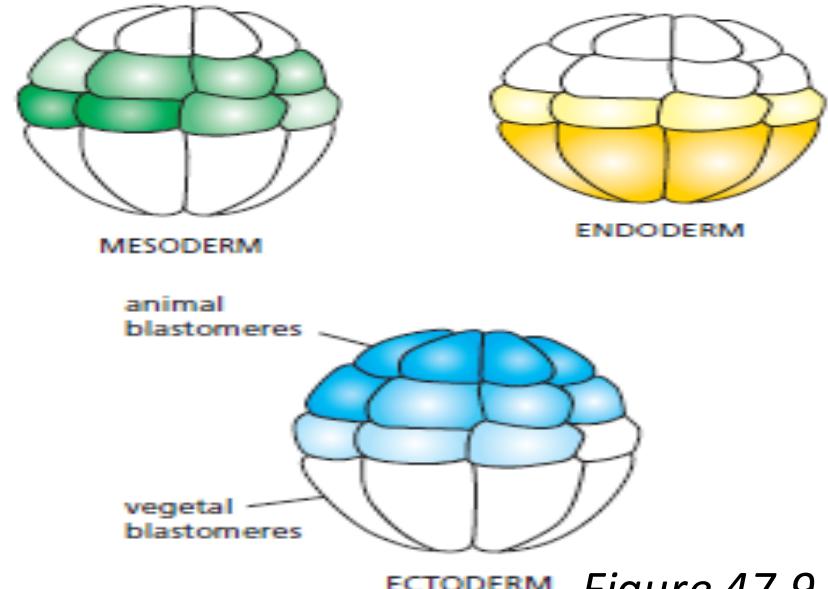
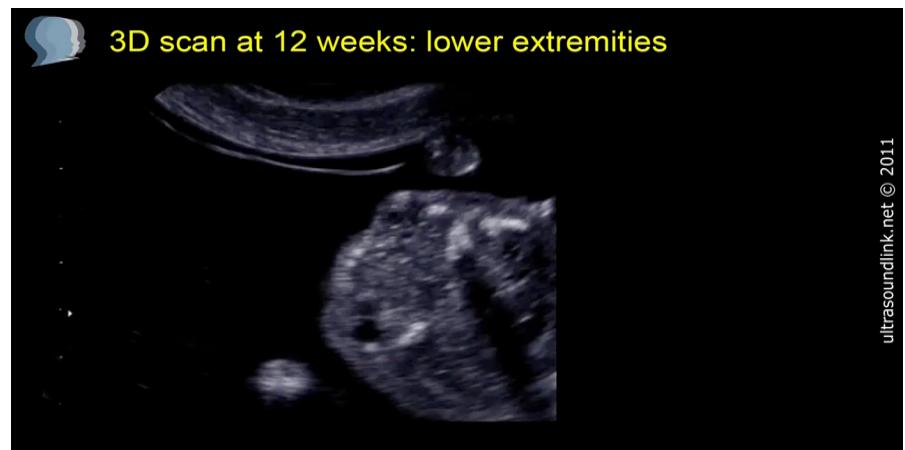


Figure 47.9



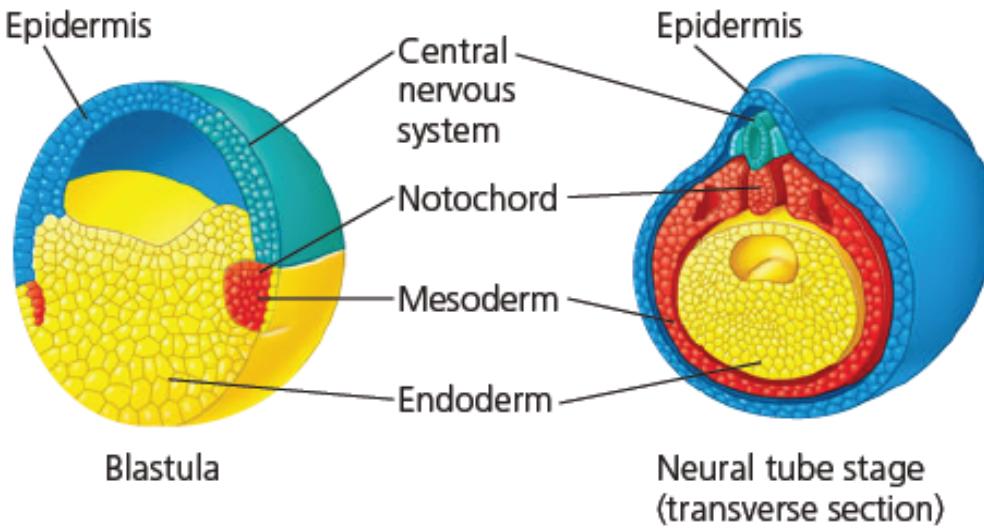
ultrasoundlink.net © 2011

Morphogenesis

- Morphogenesis - cellular & tissue-based processes by which animal body takes shape
- Last two stages of embryonic development – gastrulation & organogenesis are responsible for morphogenesis
- ***Reorganization of cytoskeleton*** associated with morphogenetic changes in embryonic tissues
- ***Programmed cell death*** or apoptosis is common feature of animal development e.g. cells in tail of tadpole undergo apoptosis during frog metamorphosis

What Determines the Fate of Cell?

- Cytoplasmic determinants & inductive signals contribute to cell fate specification

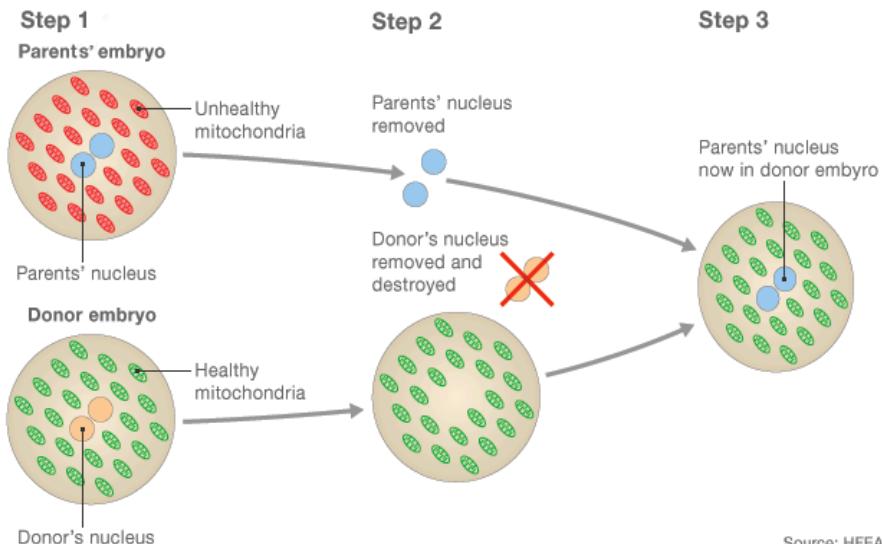


Fate map of a frog embryo *Figure 47.18*

- Fate mapping - tracing the ancestry of embryonic cells
- Experimentally derived fate maps of embryos show specific regions of zygote or blastula develop into specific parts of older embryos

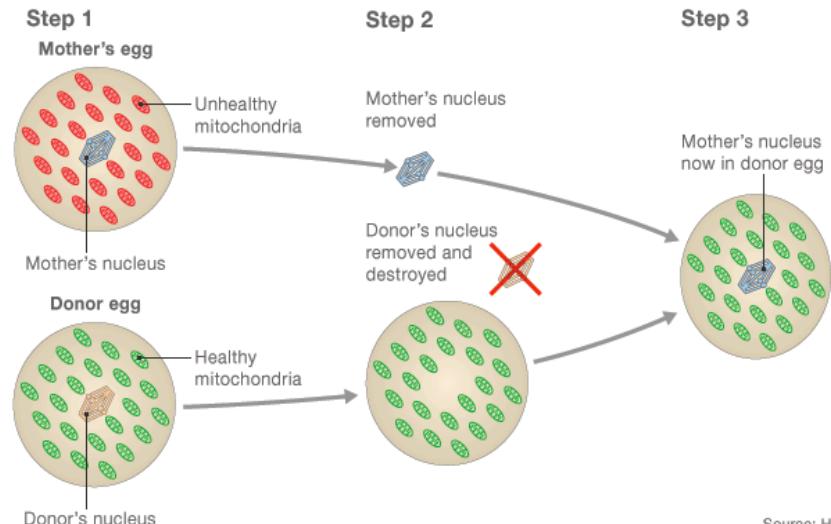
Food for thought: Three Person Babies

Method one: Embryo repair



Source: HFEA

Method two: Egg repair



Source: HFEA

UK approves three-person babies

By James Gallagher
Health editor, BBC News website

⌚ 24 February 2015 | Health

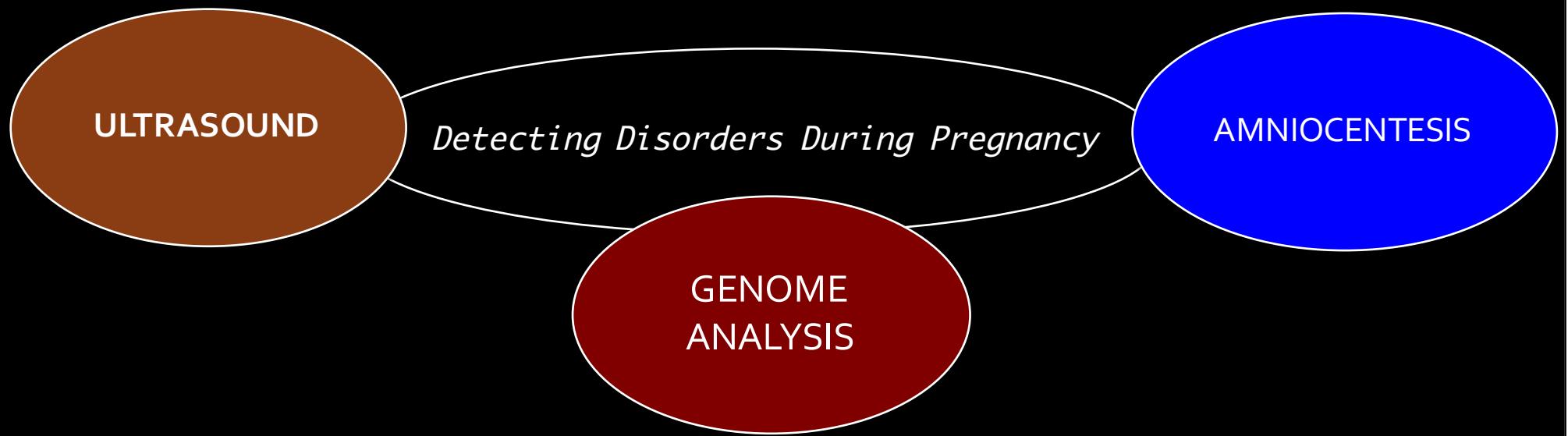
The UK has now become the first country to approve laws to allow the creation of babies from three people.

The modified version of IVF has passed its final legislative obstacle after being approved by the House of Lords.

The fertility regulator will now decide how to license the procedure to prevent babies inheriting deadly genetic diseases.

The first baby could be born as early as 2016.

Modern Reproductive Technologies



Testing a Fetus for Genetic Disorders

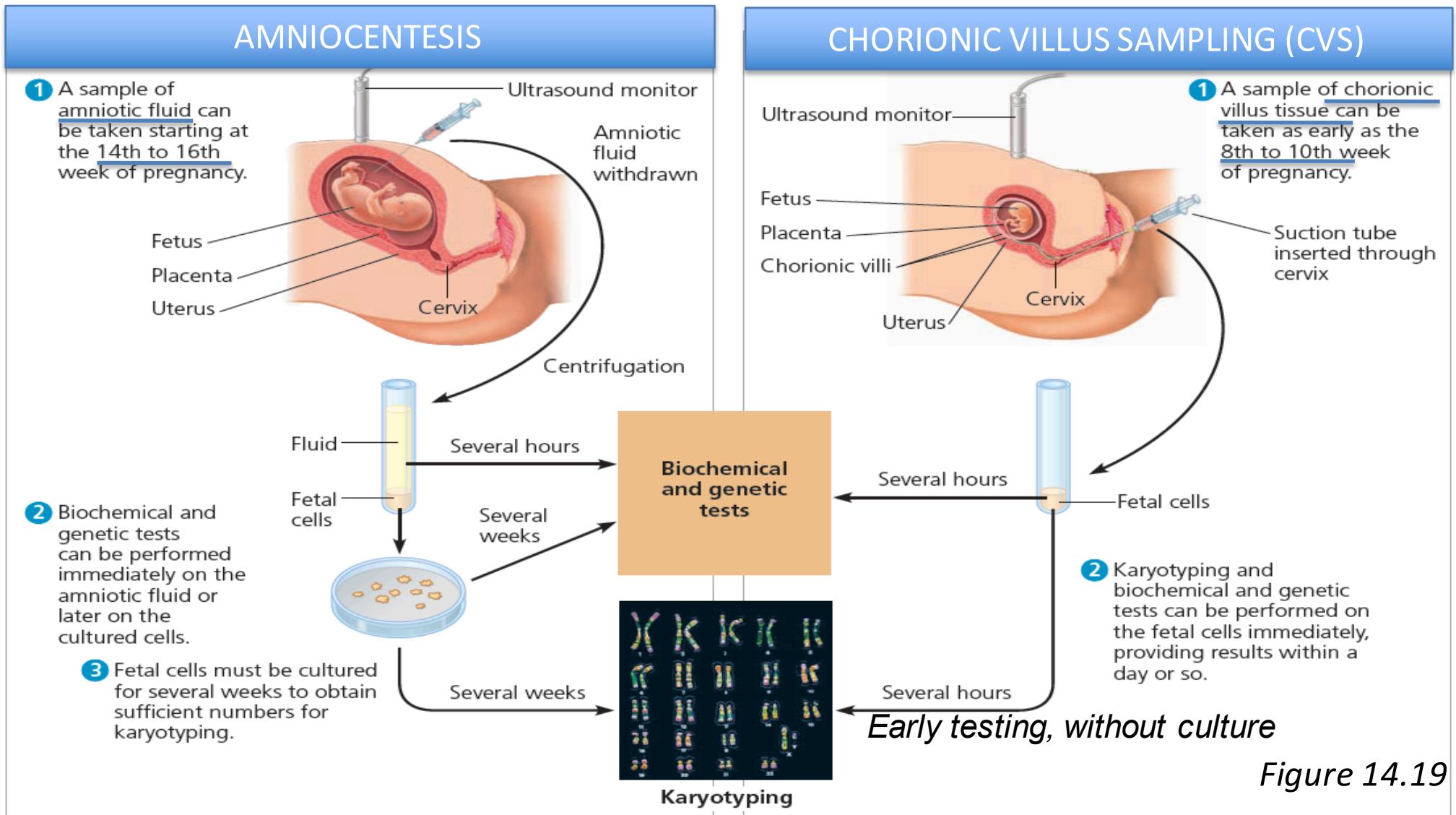


Figure 14.19

Genetic testing can detect many genetic abnormalities. Karyotyping shows whether chromosomes of fetus are normal in no. & appearance

Infertility and *In Vitro* Fertilization (IVF)

- IVF involves combining oocytes and sperm in laboratory
- Fertilized eggs are incubated until they have formed at least eight cells & are transferred to uterus for implantation



Figure 46.21

Hold the egg with a pipette and use a fine needle to inject one sperm into egg cytoplasm

- Development ✓
- Cell Reprogramming

Cell Reprogramming & Cloning

Cloning of Plants

- In plants mature cells can “dedifferentiate” and give rise to all the specialized cell types of an organism. Any cell with this potential is said to be “**totipotent**”
- Cloning plants using root cells - grow root cells in culture, cells dedifferentiate & form callus; stimulation of callus with plant hormones

Cloning of whole carrot plant from single carrot cell

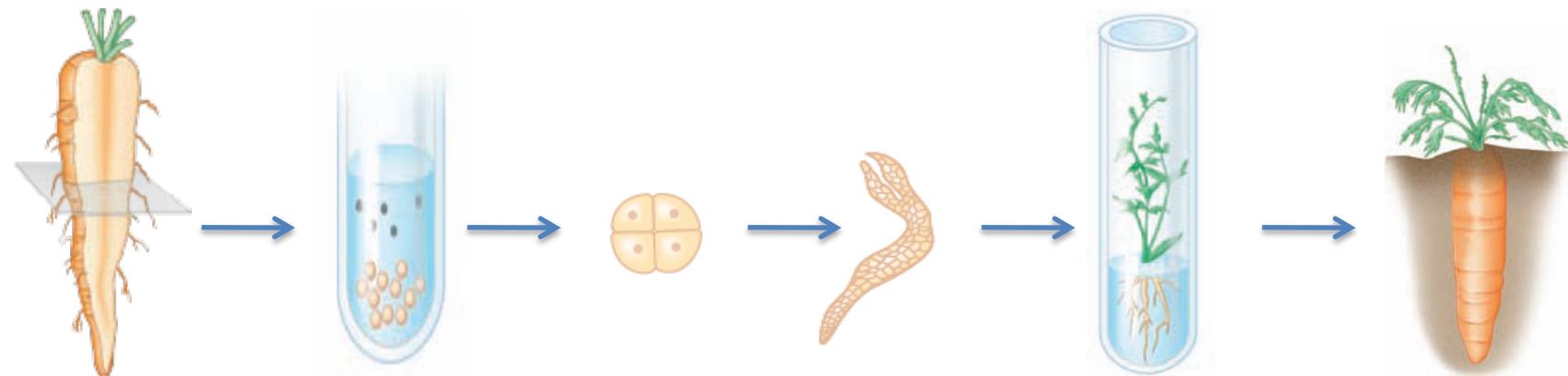


Figure 20.15

18

Cloning of Plants (2)

- Plant cloning has many applications in agriculture.
- Interestingly, plants such as orchids are commercially produced by cloning only.
- Cloning has also been used to reproduce a plant with valuable characteristics, such as resistance to plant pathogens.
- Have you ever cloned a plant?
 - *If you have ever grown a new plant from a cutting, you have practiced cloning!*

Cloning Animals: Nuclear Transplantation

Are differentiated animal cells totipotent?

Scientist removed nucleus of an unfertilized or fertilized egg and replaced it with nucleus of a differentiated cell, this procedure is called “nuclear transplantation”

If nucleus from differentiated donor cell retains its full genetic potential, then it should be able to direct development of recipient cell into all the tissues and organs of an organism.

Gurdon's experiment

- Gurdon destroyed nuclei of frog (*Xenopus laevis*) eggs by exposing eggs to UV light. Transplanted nuclei from cells of frog embryos & tadpoles into enucleated eggs

- Results:
 - (a) when transplanted nuclei came from an early embryo, most recipient eggs developed into tadpoles
 - (b) when nuclei came from fully differentiated intestinal cells of a tadpole < 2% eggs developed into normal tadpoles

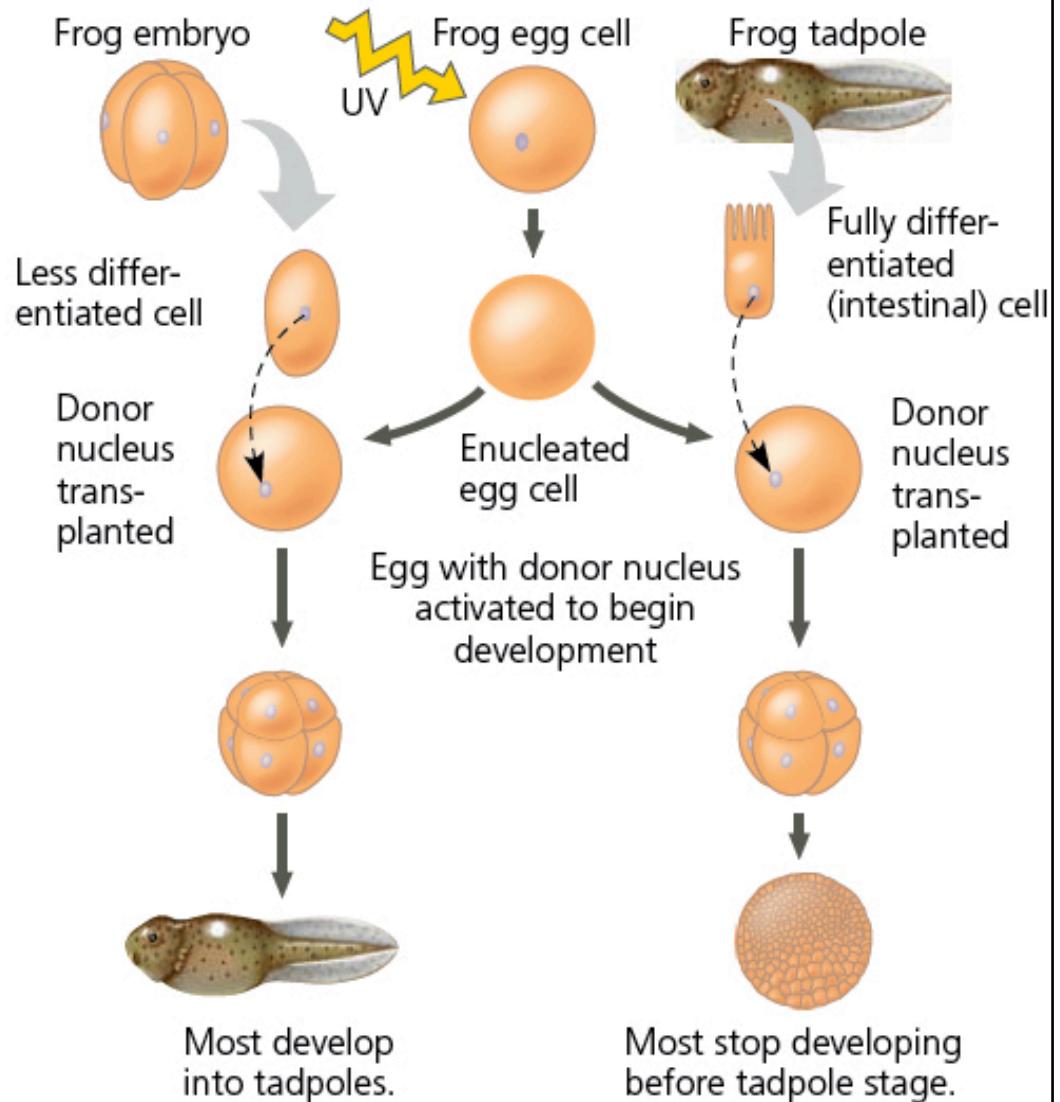


Figure 20.16

Reproductive Cloning of Mammals

Scientists had shown that they could clone mammals by transplanting nuclei or cells from variety of early embryos into enucleated eggs.

However, it was challenging whether a nucleus from a fully differentiated cell could be reprogrammed successfully to act as a donor nucleus.

Scientific fiction transformed into Reality?

*1997 Dr. Ian Wilmut of Roslin Institute cloned a sheep
“Dolly” - First large cloned animal from somatic cells*

These researchers achieved dedifferentiation of donor nuclei by culturing mammary cells



Cloning Animals

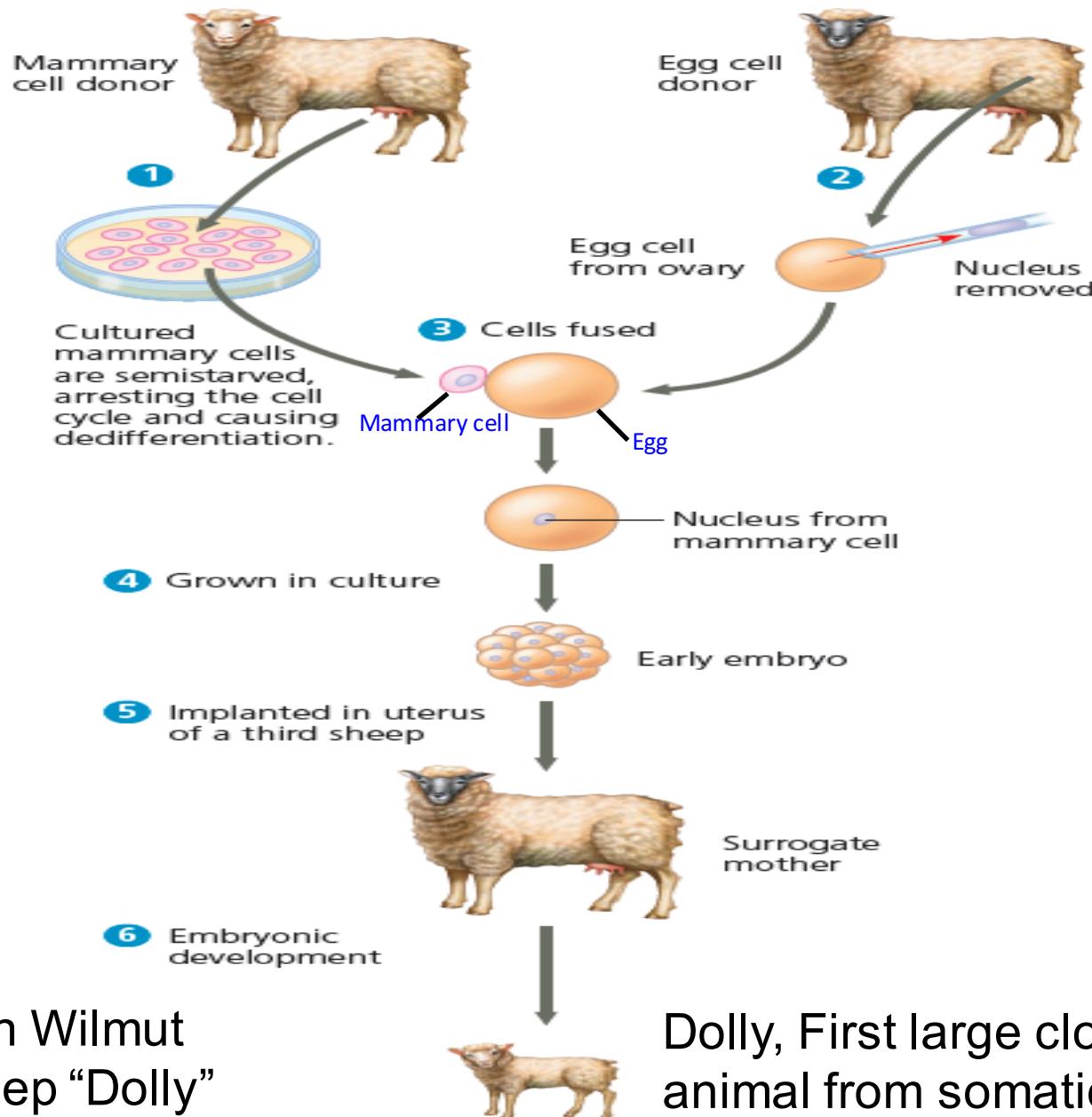
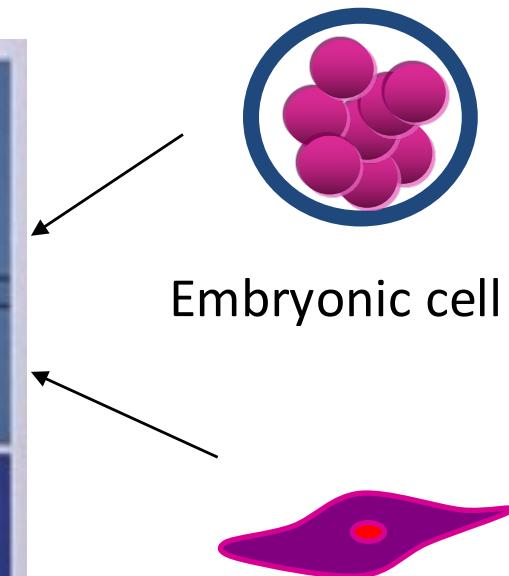
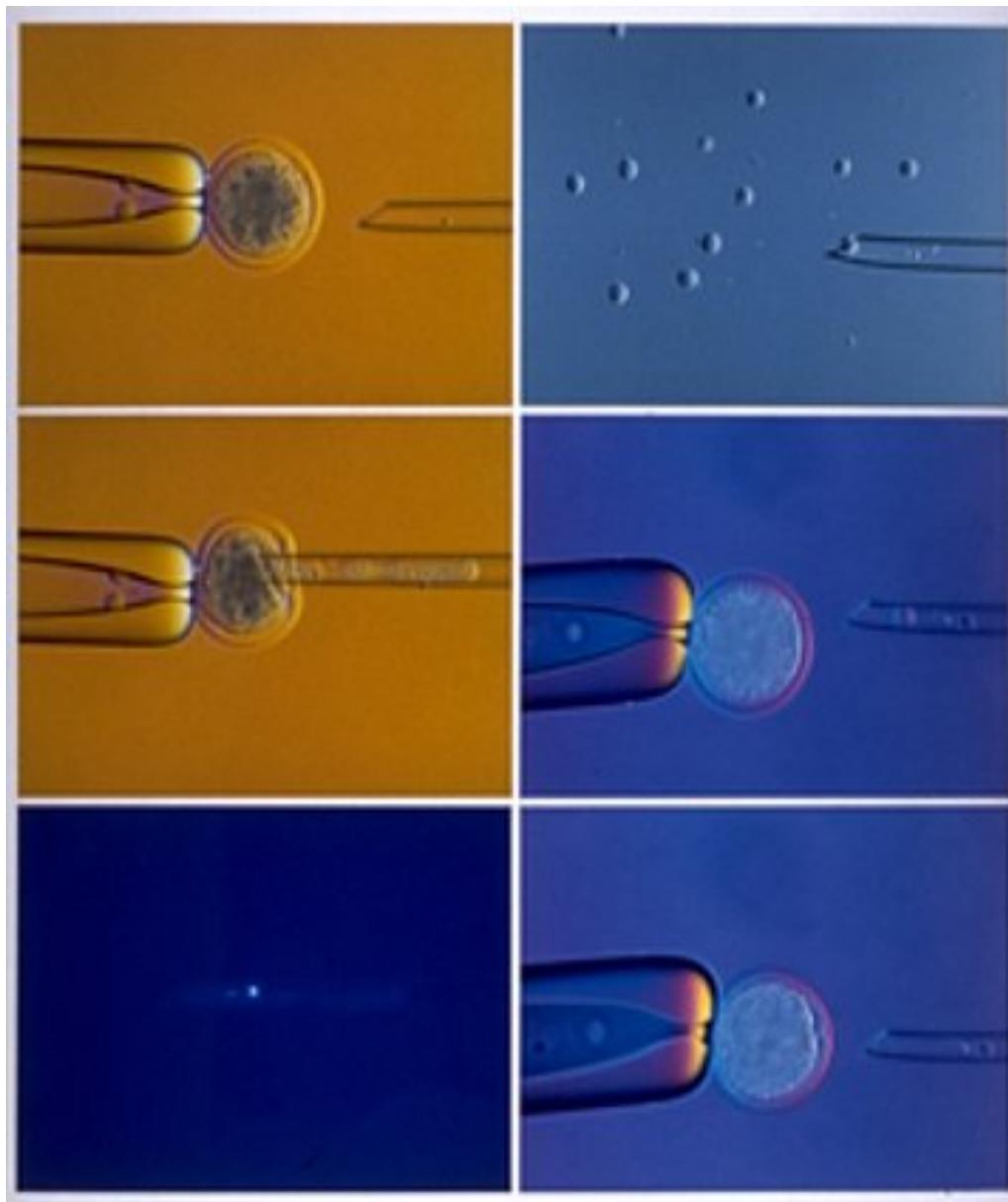


Figure 20.17

- 1997 Dr. Ian Wilmut cloned a sheep "Dolly"

Cloning Animals: Mechanism



Cloned animals: do they always look same?

No

e.g. Cows cloned from same line of cultured cells - certain cows showed dominant behavior and others were submissive.

CC Cat: first Cloned Cat

- Nucleus was taken from Rainbow (left cat) having classic calico feature with orange patches on her fur, and cloning was performed.
- CC (right) is a cloned cat but it has gray and white coat
- Therefore, two cats were not identical



Figure 20.18

Possible reason for difference could be due to random X chromosome inactivation, which is a normal occurrence during embryonic development

Identical Human Twins: are they exactly same?

Even naturally occurring “clones,” the “twins” are always slightly different.

It clearly demonstrates that environment influences and random phenomena play a significant role during development.

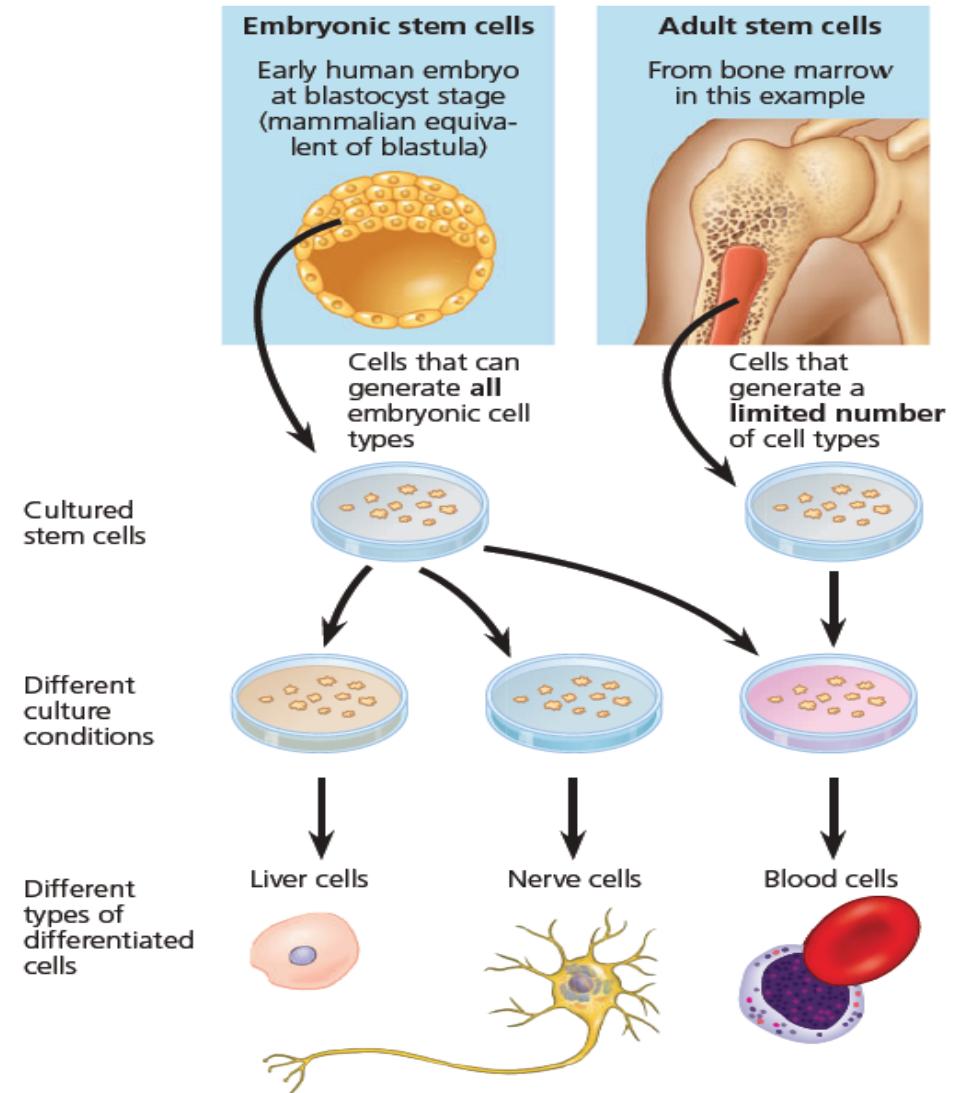
Faulty Gene Regulation in Cloned Animals: Ethical Concerns!

- In nuclear transplantation studies, majorly, only small percentage of cloned embryos developed normally; low efficiency of cloning & high incidence of abnormalities
- Epigenetic and genetic mechanisms are involved in clone failure, but we don't know exactly how?
- Reprogramming of donor nuclei requires more accurate and complete *chromatin restructuring*
- Identification of mechanisms that underlie developmental abnormalities caused by cloning is required

A tale of landmark studies of stem cell research: *biggest prize and biggest scientific scandals*

Embryonic Stem Cell

- ES cells can be derived from early human embryos & fetal germ cells
- ES cells can be induced to differentiate into a wide variety of cell types in culture, by treatment with combinations of signal proteins & growth factors
- The major goal of cloning human embryos is not reproduction but to produce stem cells to treat human diseases



Nobel Prize - 2012

- Theme: Discovery that mature cells can be reprogrammed to become pluripotent



Sir John B. Gurdon



Dr. Shinya Yamanaka

- *The biggest challenge, direct reprogramming by defined factor was resolved by the basic research*

The Rise and Fall of Hwang Woo-Suk



FEBRUARY 2004

Woo Suk Hwang describes the first stem-cell line, NT-1, derived from a cloned human embryo.

MAY 2005

Hwang's group publishes a second paper reporting 11 further human embryonic cell lines.

AUGUST 2005

Hwang's group is the first to clone a dog.

NOVEMBER 2005

US collaborator Gerald Schatten splits with Hwang, citing ethical problems in getting human eggs.

DECEMBER 2005

Pushed by increasing evidence, Seoul National University (SNU) launches an investigation.

JANUARY 2006

Hwang's human-cloning research is deemed fraudulent by SNU. His dog-cloning claims are upheld.

JULY 2006
Sooam Foundation starts up, with US\$3.5 million from Hwang's supporters.

2007
The Korean health ministry grants Sooam the right to do human-embryo and cloning research.

OCTOBER 2009
Hwang is found guilty of embezzlement and bioethics violations. Appeal continues.

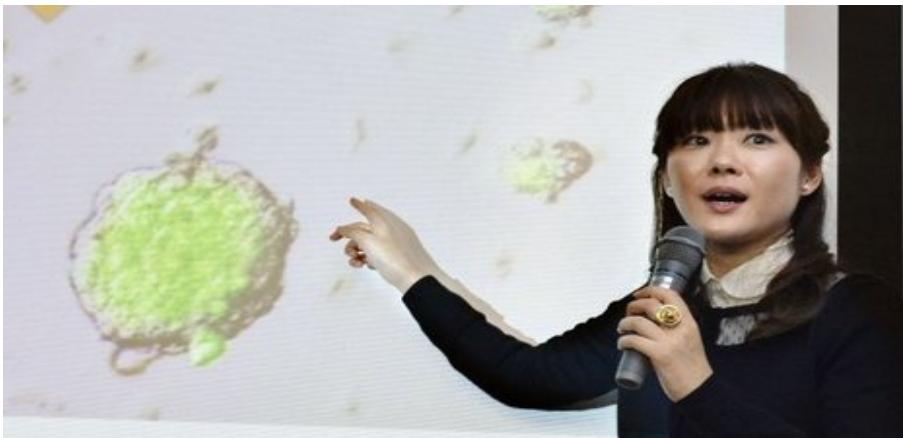
2011
Canada grants Hwang a patent for the NT-1 cell line.

2012
Sooam scientists clone a coyote using a dog egg-cell donor and surrogate mother.

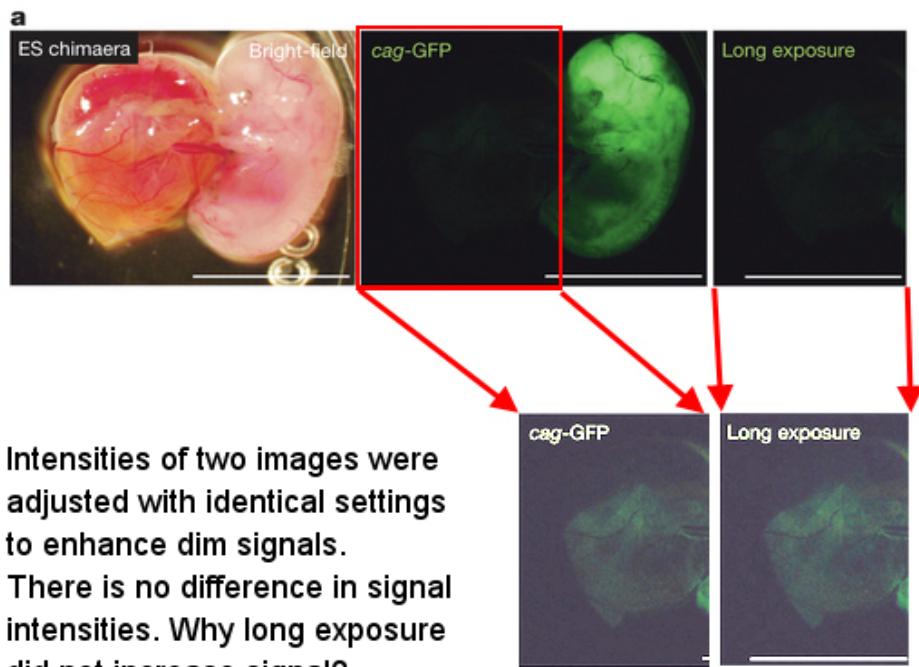
2013
Court tells the Korean Centers for Disease Control and Prevention to register the NT-1 cell line.



Dr. Haruko Obokata



Reported a cellular reprogramming phenomenon, STAP (Stimulus-triggered Acquisition of Pluripotency), which requires neither nuclear transfer nor introduction of transcription factors



Intensities of two images were adjusted with identical settings to enhance dim signals.
There is no difference in signal intensities. Why long exposure did not increase signal?

The screenshot shows a news article from the journal 'nature'. The header includes the word 'nature' and 'International weekly journal of science'. Below the header is a navigation bar with links: Home, News & Comment, Research, Careers & Jobs, Current Issue, Archive, Audio & Video, and For Authors. A secondary navigation bar shows the path: News & Comment > News > 2015 > March > Article. The main content is titled 'Moot punishments for Japanese STAP scientists'. The text below the title states: 'Lead author of retracted stem-cell papers is fired, and a co-author is suspended, from jobs they no longer held.' The author's name, David Cyranoski, is mentioned. At the bottom, the date is given as 10 February 2015 | Updated: 11 February 2015.

Cell Reprogramming: Rise and Fall of Stars



Sir John B. Gurdon



Dr. Shinya Yamanaka
Nobel laureates



Dr. Haruko Obokata



Dr. Hwang Woo-Suk
Fraudulent research

Ethics in Research & Publication

Summary

- Fertilization and cleavage initiate embryonic development
- Modern reproductive technologies help in detecting disorders during pregnancy
- Common theme for all cloning: product is identical to parent
- The biggest landmark studies in stem cell research resulted into biggest prize as well as biggest scientific scandals (opens discussion for “*ethics in research and publications*”).

References

- Campbell Biology - Reece, Urry, Cain, Wasserman, Minorsky, Jackson 10th Edition, Pearson
- Wilmut, I., et al., Nature, 385: 264-267 (1997)
- *Acknowledgment*
 - Cover images – getty images



Next Lecture...

Genetics

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