

# 4-H Curriculum Training Embryology

August 21, 2012



Welcome



POULTRY SCIENCE



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## Who we are:

### Department of Poultry Science

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### South Central District – Iredell

> Kathy Bunton – Area Poultry Agent; [ssbunton@ncsu.edu](mailto:ssbunton@ncsu.edu); 704-878-3154

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## 4-H Curriculum Training

### You have to have a better understanding than those you are teaching

- Incubator setup and understanding the operation
- Egg Formation
- Shell properties
- Embryology – Normal development and understanding of extra-embryonic membrane formation
- Lab: Embryo candling & breakout
- Incubation Parameters
- Problem solving hatch
- Lab: Incubation parameters & chick quality

### Experiments and understanding

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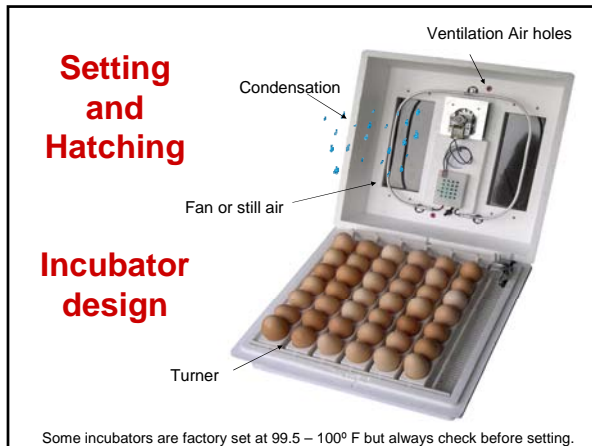
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**Egg Storage and Setting for Optimal Hatchability**

- ✓ Don't store eggs for longer than a week if possible
- ✓ Set and store eggs small end down
- ✓ Store eggs at approximately 55°F
- ✓ Humidity in egg cooler approximately 75%
- ✓ Don't set dirty eggs – bacterial contamination

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**Hygrometer for temperature and humidity regulation**

**Breakout poster is helpful to determine embryo growth.**

[http://www.strombergschickens.com/product\\_print/High-Accuracy-Thermometer-Hygrometer/incubator-hygrometers](http://www.strombergschickens.com/product_print/High-Accuracy-Thermometer-Hygrometer/incubator-hygrometers)

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### Embryonic Development in the Chick

- Day 1 – Primitive streak, somite
- Day 2 – Heart beat, blood circulation
- Day 3 – Amnion encircles embryo
- Day 4 – Eye pigment, leg bud larger than wing
- Day 5 – Joints appear on legs and wings
- Day 6 – Beak and toe formation
- Day 7 – Comb and egg tooth appear
- Day 8 – Prominent feather tracts
- Day 10 – Toe nails appear
- Day 11 – Comb serrated, tail feathers
- Day 13 – Down cover, scales on legs
- Day 15 – Small intestines taken into abdomen
- Day 16 – Feather cover
- Day 17 – Head between legs
- Day 18 – Head under right wing
- Day 19 – Amnionic fluid disappears, yolk sac half withdrawn
- Day 20 – Yolk sac drawn into body, beak pips through membrane
- Day 21 – Shell is pipped, chick hatches

Jenna L. Wilson, Department of Poultry Science, The University of Georgia, Athens, GA

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### Incubator setup and understanding the operation

- **Temperature** – Dry bulb approximately 99.5°F during the setting period then 98.5°F for hatching usually (3 days before hatching).
- **Humidity** – different at setting and hatching
  - Wet bulb – measure of evaporative cooling effect - 82 to 84°F at set
  - Relative Humidity – 48-53% for setting and approximately 60% at hatching

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### Incubator setup and understanding the operation

- **Turning**
  - Automatic, continuous motor
  - Hand turn - couple times a day, mark egg; turn eggs from set day to 3 days before hatching, then remove turner.
- **Ventilation – observe when to open vents:**
  - Supplies oxygen, removes water vapor, carbon dioxide and metabolic heat
  - Condensation – don't let it bead up

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**If temperature is not preset then set to the correct temperature. Calibrating with 2 thermometers is best!!**

- **TEMPERATURE REGULATION:** Establish a temperature of 99.5° F. First, turn the control knob fully clockwise. You will see the red indicator light come on. The element in the top half will start to heat. Watch the temperature as the heat comes up. When the temperature has reached about 99 degrees, begin to gradually turn the control knob slowly counterclockwise. You may need to move the control back and forth until you achieve the recommended temperature.
- **CLOCKWISE—INCREASE HEAT. COUNTER-CLOCKWISE—DECREASE HEAT.** The red indicator light will probably flicker which it should. The flickering indicates that the incubator is adjusting to changes in both internal and external temperatures.
- **Run the incubator about 8 hours before eggs are set.** Observe the temperature and at the end of this adjustment period, make sure the temperature is where you want it to be.

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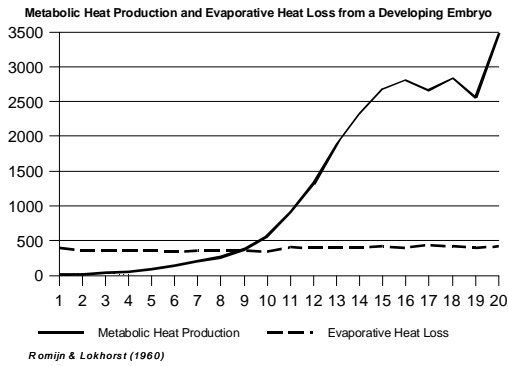
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**Heat Production by Embryo**



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**Humidity in the Incubator**

- ❖Governs moisture loss from the egg.
- ❖Typically eggs will lose 10-14% of weight over an 18 day period as moisture.

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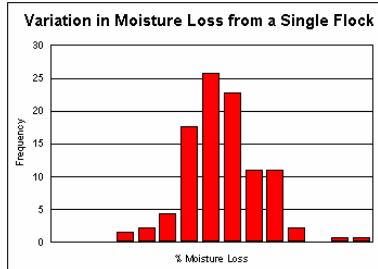
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## Humidity - Moisture Loss

- Varies within flocks 7-18% at 18 days of incubation.



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## Turning

- Eggs start in the incubator in the vertical position, large end up!
- Turning angle of the egg is typically 45 degrees from vertical
- Happens in two directions from vertical



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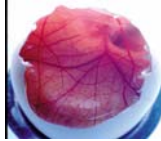
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## Turning



- Failure to turn- common belief - embryo adheres to membranes
- More complicated
  - day 3-7 rapid development
    - Influences growth of the Chorio-allantois Membrane (**CAM**)
    - Influences growth of the yolk sac
    - Influences formation of sub-embryonic fluid
  - Turn through day 13 at minimum

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## Ventilation

- Control oxygen and carbon dioxide levels
  - egg influence (pores)
  - machine influence (pressures, humidity)
  - remember - high to low concentrations
- Internal circulation
  - uniform temperature
  - remove metabolic heat
- Controls relative humidity

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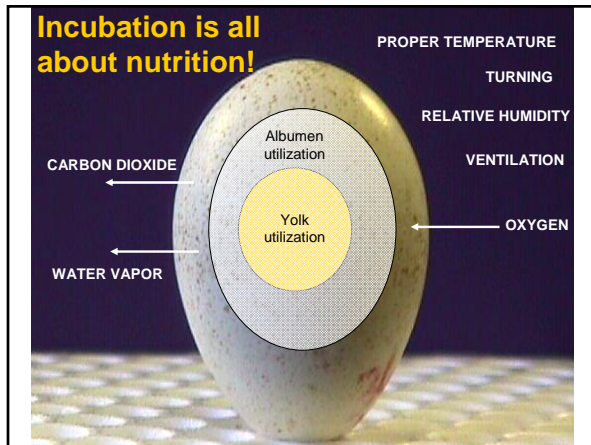
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Artificial insemination of a turkey breeder hen



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Chickens – average semen volume is 0.5-1.0 ml with semen concentration from 1.7-3.5 million/ml respectively.



Turkeys – average 0.2 ml per collection with concentration ranges from 6.2 to 7 million/ml

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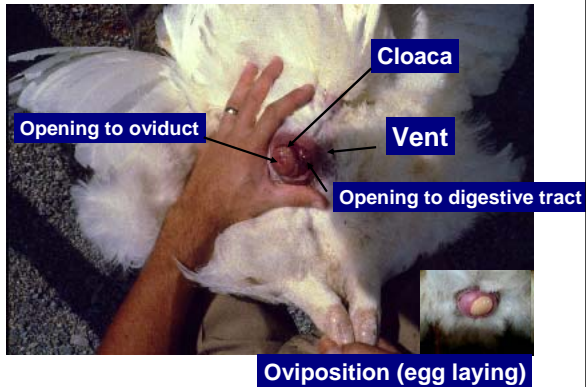
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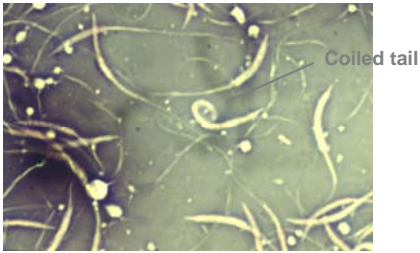
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### Sperm Cells



Birds are polyspermy which means the entry of several sperm into one ovum during fertilization but only one will fertilize the egg.

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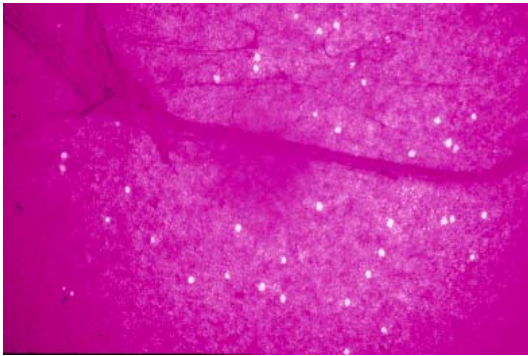
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### Inner Perivitelline Layer Sperm Penetration Holes



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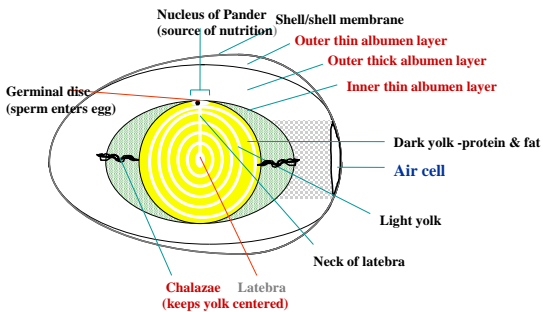
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### Structure and Physiology of an Egg



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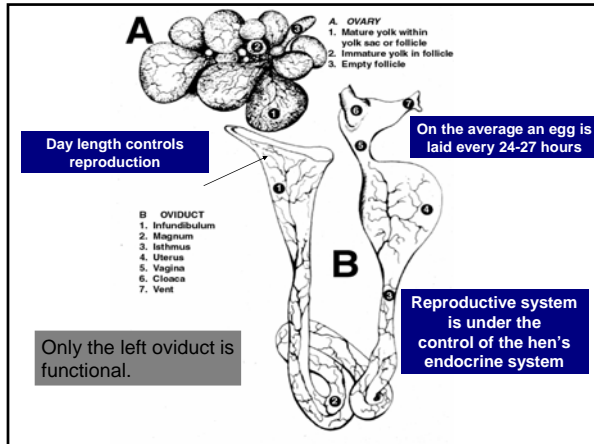
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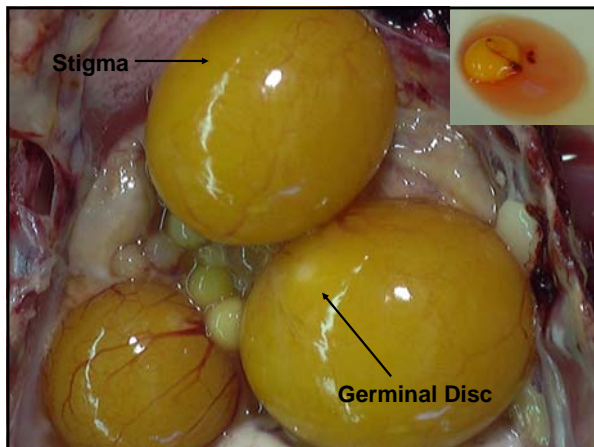
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### Follicular hierarchy in the laying hen



**Ovum** – if the ovum fails to be picked up by the **Infundibulum**, then it is reabsorbed. Fertilization occurs and the ovum remains here for only 15-30 minutes.

*Sperm storage glands*

Infundibulum-magnum junction

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**Magnum** – is the largest part of the oviduct, about 13 inches in length. The ovum remains here for 2-3 hrs and moves by peristaltic motion. Albumen is secreted here.




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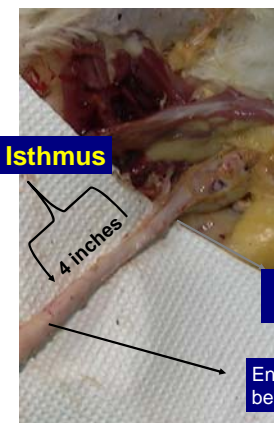
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**Isthmus**

4 inches

Both inner and outer shell membranes are formed during the 1-2 hr passage . Separation of these membranes form the **air cell**.

End of Isthmus and beginning of **uterus (shell gland)**

End of Magnum and beginning of the Isthmus

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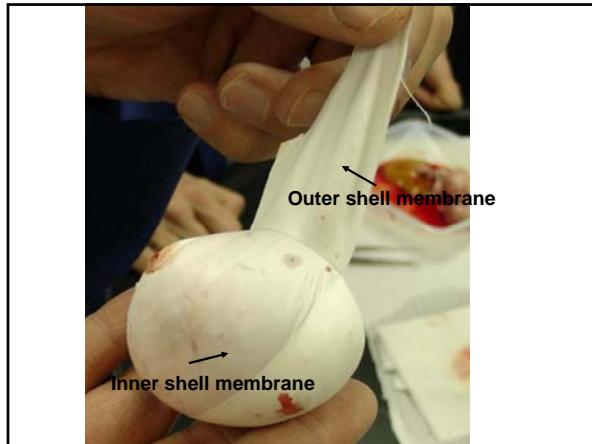
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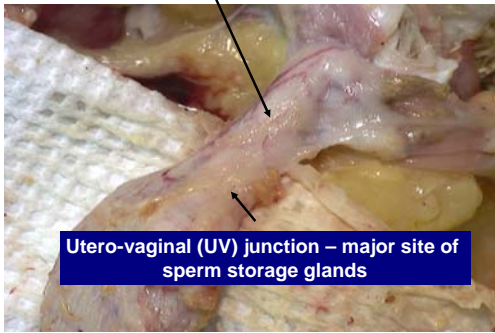
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**Vagina** – functions to expel the egg with help from the shell gland (uterus) and is the site of insemination



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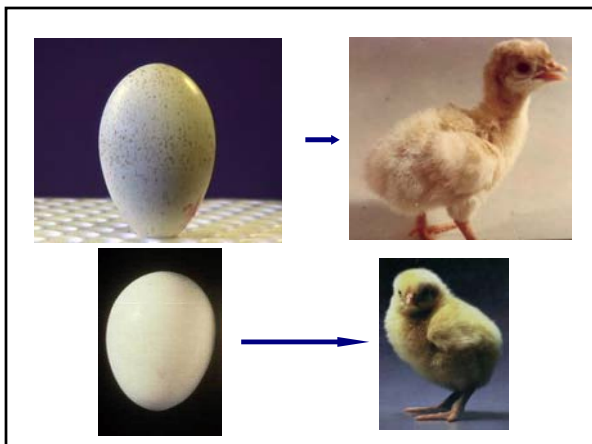
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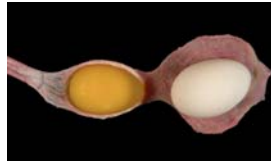
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### Slab Sided Egg

- Two eggs in the shell gland
- Ovulated within a 24 hr period



Dr. John Barnes, College of Veterinary Medicine, North Carolina State University

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### Rough, Wrinkled or Misshapen Shells



- Newcastle or Bronchitis
- Calcareous deposits

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### Causes of Thin shells



- Shells become thinner as temperatures go above 70 °F
- Older birds lay eggs with thin shells
- Birds lay two eggs within 24 hrs.

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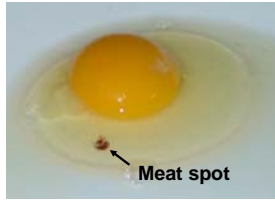
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### BLOOD & MEAT SPOTS



- Blood vessel rupture at stigma line
- Sloughing of oviduct tissue

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### DOUBLE YOLKS

Common when egg production begins; young hens



Regular rhythm hasn't developed yet

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### MOTTLED YOLKS

Mottling-  
Water trapped between the vitelline membrane layers



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## Shell properties

- How egg breathes
  - Passive diffusion
    - high concentration to lower concentration

Gases and water move across the shell.  
(O<sub>2</sub>, CO<sub>2</sub> and H<sub>2</sub>O)



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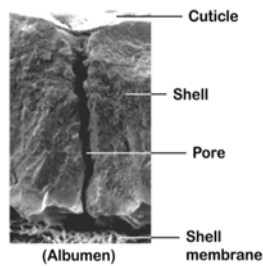
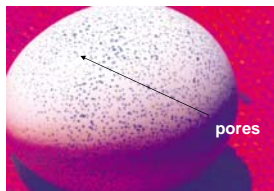
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- Water loss (which can occur through the pores) is mandatory. Without it, hatching would not occur. Air cell forms with the water loss.

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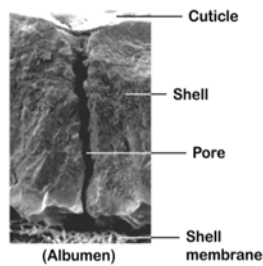
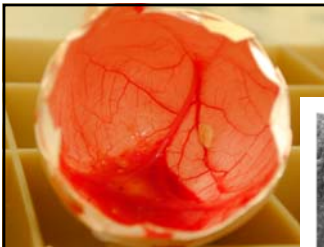
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Chorio-Allantoic Membrane (CAM) is responsible for respiration.

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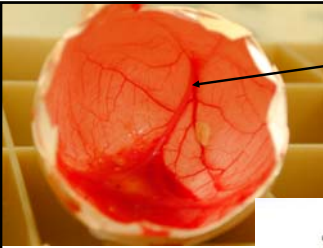
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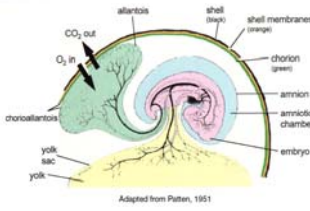
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**Chorio-Allantoic Membrane - CAM**

- Fat is the major nutrient metabolized
- Water is approximately 67-70% of fresh egg and new hatched chicks should be the same




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## **Embryology**

- Embryology – Understanding of normal development, growth and formation of living organisms.
- Working with BIOLOGY = Variation

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## **When does embryo development (incubation) start??**



Lets take a look at embryonic development in the chicken!!

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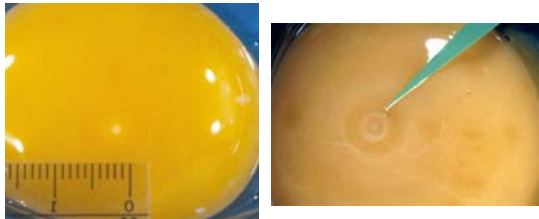
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## Fertile or Infertile???



Embryo starts developing rapidly about 4 hrs after fertilization

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## Candling Eggs for Fertility Assessment

11 day embryo – usually done a week after set



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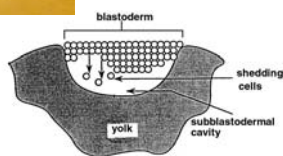
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## Fertile Egg



- At oviposition (also known as the process of the egg being laid).



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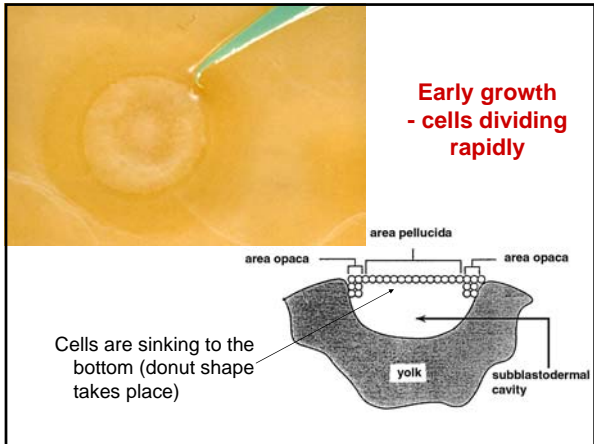
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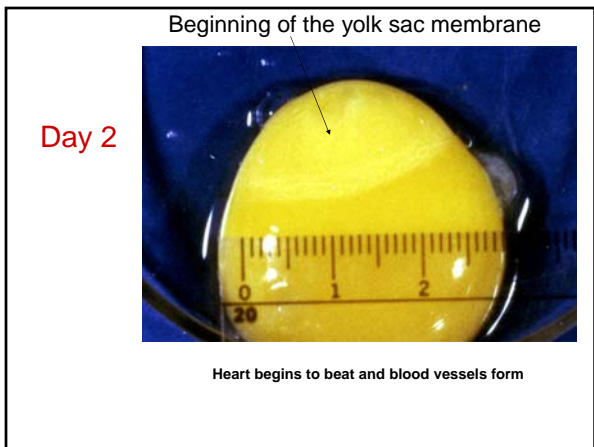
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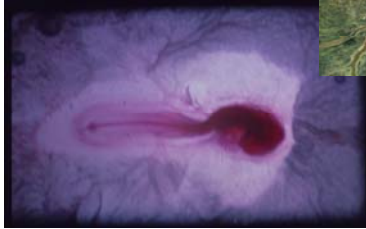
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Day 3



Paired Somites  
– somites  
develop into  
internal organs

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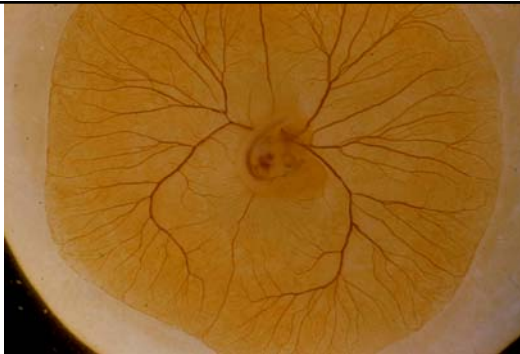
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Day 3 The vascularized yolk sac membrane is formed (area vasculosa).

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Heart Rate will decrease as the egg cools so it is very important to keep the egg warm at all times. Heart beat can be detected as early as day 12 in a turkey and day 10 in a chicken



Initial Heart Rate \_\_\_\_\_



Heart Rate 30 minutes later \_\_\_\_\_

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## Day 4

- Embryo lays on left side on top of yolk
- Rapid development of the CNS
- Water movement from albumen into yolk - albumen sinks because of resulting change in specific gravity



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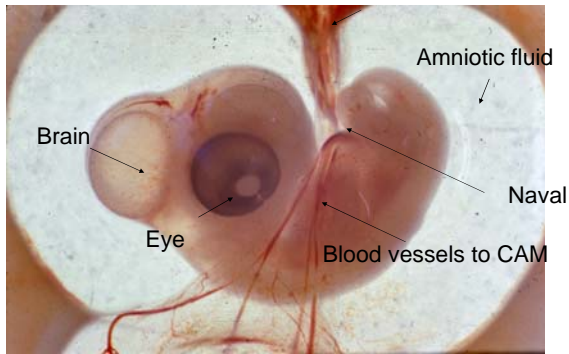
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## Day 6

### Blood vessels to yolk sac



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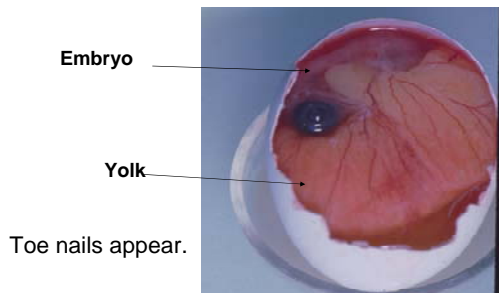
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## Day 10



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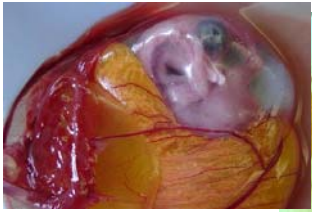
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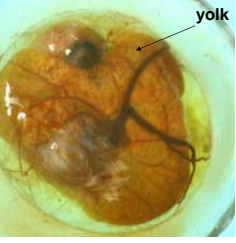
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Day 11



Albumen begins to be absorbed in amniotic cavity via sero-amniotic duct.

Day 13



Embryo sinks into the yolk, head in large end of egg

albumen

yolk

Embryo lies across the yolk

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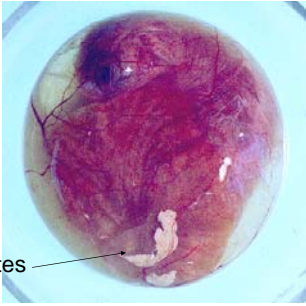
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Day 16

- **Urates (insoluble urine) - Excretions from embryonic kidneys**
  - Primarily is uric acid that increases as development proceeds.
  - Birds don't urinate; the white droppings in fecal is uric acid.

Urates become evident in the Allantois (water reservoir), albumen nearly all gone.



Urates

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Movement to hatching position



18d hbl      19 d urw      20 d urw

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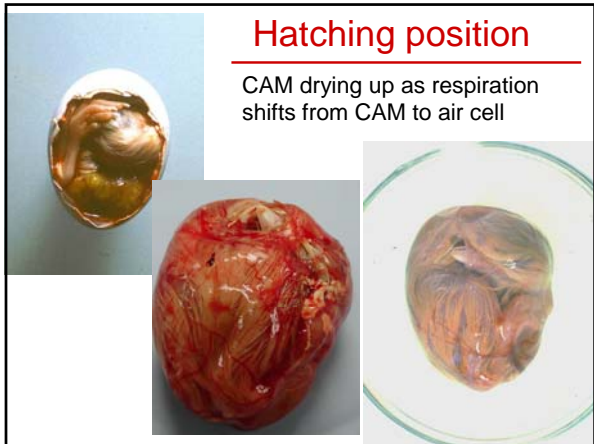
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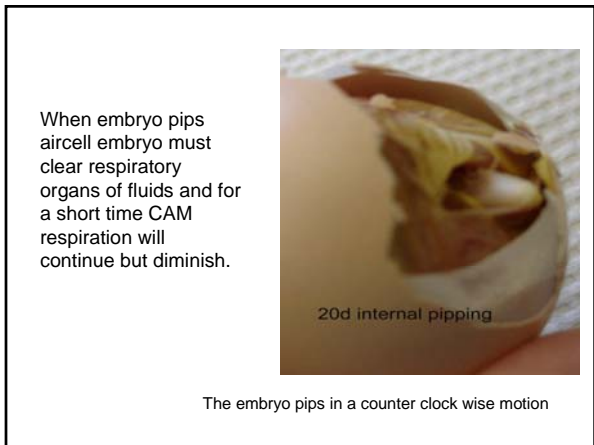
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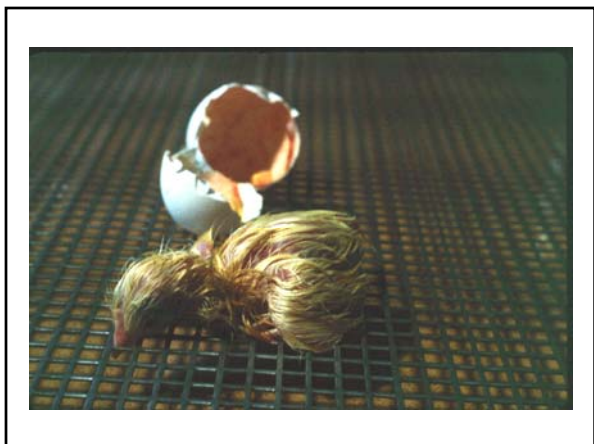
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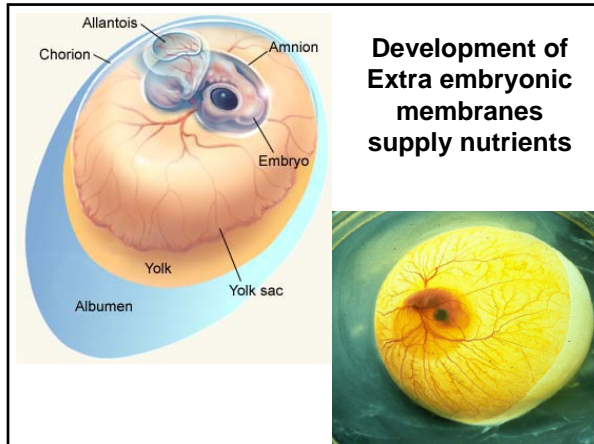
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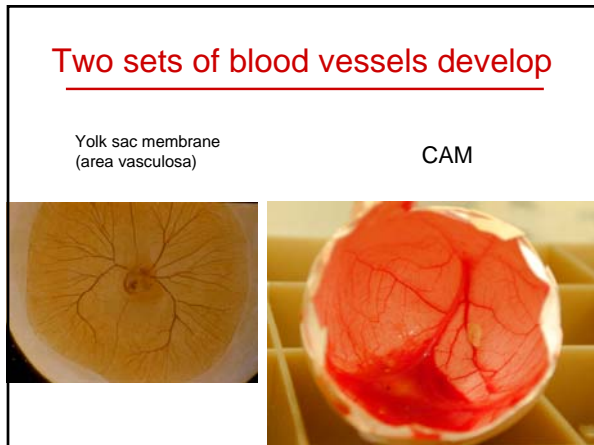
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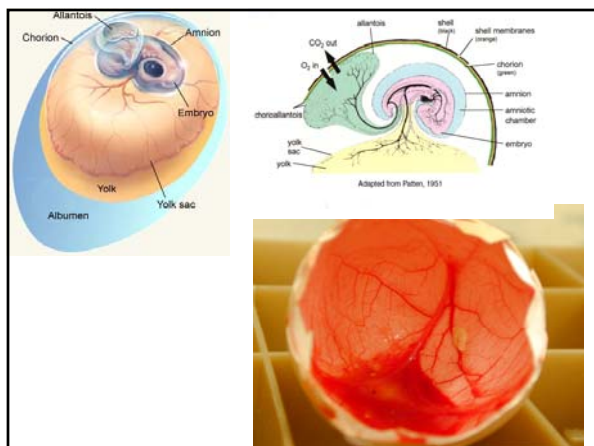
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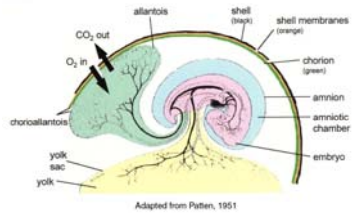
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## Extra-Embryonic Component

- Membranes
  - **Yolk sac**
  - Amnion
  - Chorion
  - Allantois
- Sub-embryonic fluid



Adapted from Patten, 1951

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## Yolk sac membrane

- **Vitelline membranes** enclose yolk until day 4 then vitelline membrane slips down to vegetal pole
- Yolk bounded by **yolk sac membrane**
  - Area Vasculosa
  - Nutrient absorption
  - Initial respiratory surface



72 hrs.

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## Yolk sac membrane

- Day 12 yolk sac becomes 3 flabby lobe mass
- Finally surrounds yolk at 14-15 days



lobes

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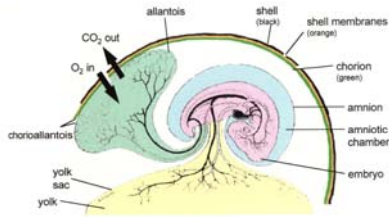
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## Extra-Embryonic Component

- Membranes
  - Yolk sac
  - **Amnion**
  - Chorion
  - Allantois
- Sub-embryonic fluid



Adapted from Patten, 1951

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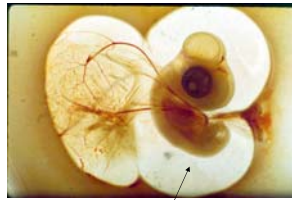
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## Amnion & Amniotic fluid

- transparent fluid filled sac
  - protect from shock
  - antibacterial properties



Amniotic fluid

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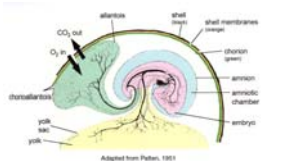
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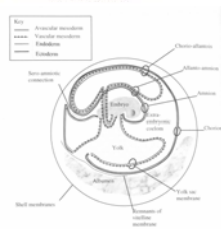
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## Extra-Embryonic Component

- Membranes
  - Yolk sac
  - Amnion
  - **Chorion**
  - **Allantois**
- Sub-embryonic fluid



Adapted from Patten, 1951




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## Allantois and Chorion

- **Vascularized when contact with chorion** (called chorio-allantoic membrane (CAM), lines inner shell - respiration and absorption of calcium
- **Fluid filled** (from umbilicus) involved in **water conservation**
- Aids in albumen utilization

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## Allantois

- **Excretions from embryonic kidneys**
  - Excretory nitrogen primarily as uric acid
- **Incubation temperatures**
  - Fluid accumulates more rapidly at elevated temperatures
  - Fluid accumulates more slowly at sub optimal temperatures

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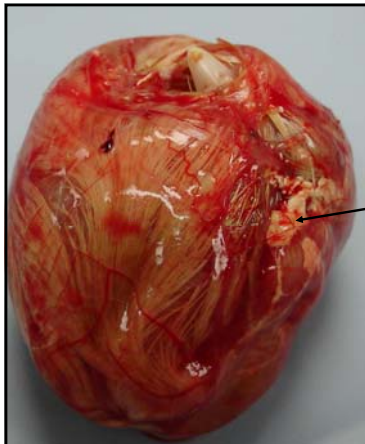
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**CAM** exposed in a internally pipped embryo (around day 19-20)

**Urates** (wastes from the kidneys) are deposited in the **allantois sac** (water reservoir)

**Yolk sac** – should be almost internally absorbed by now

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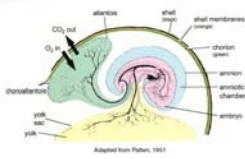
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## Extra-Embryonic Component



- Membranes
  - Yolk sac
  - Amnion
  - Chorion
  - Allantois
- Sub-embryonic fluid (develops within upper part of yolk)

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## Sub-embryonic fluid - SEF

- A fluid which appears in the yolk sac beneath the embryo (movement of water from albumen into the yolk plus K<sup>+</sup>, Na<sup>+</sup>)
- Critical period for production of SEF
  - 3-7 days



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## Sub-embryonic fluid

- Formed by water moving into the yolk sac which makes the yolk lighter than albumen and causes it to float to upper surface of egg
  - thus [area vasculosa](#) is close to shell membranes
    - improving embryo access to air easier early in incubation



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## Sub-embryonic fluid

- Critical period for production of SEF
  - 3-7 days
    - CO<sub>2</sub> influence
    - Absence of turning reduces growth of area vasculosa over yolk sac and causes reduced SEF

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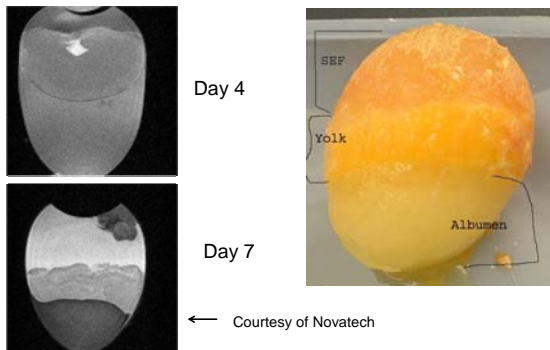
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## Sub Embryonic Fluid Formation



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## Embryology Lab

- Embryo candling
- Embryo breakout

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## Embryo Growth

- The *Rate of Growth* of an embryo is driven by temperature
- The *Growth* of an embryo is driven by oxygen

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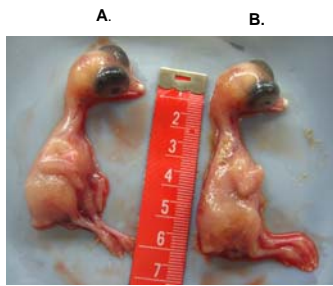
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Incubating eggs at hotter temperatures can cause the embryo to develop faster in the early stages of incubation, but only until several days before hatch when they slow down.



Which turkey embryo was incubated at the higher temperature?

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## Incubation Problem Solving

**Temperature, turning, humidity, and ventilation** all effect the outcome of your hatch causing abnormal embryo growth.

It is very important to break out all unhatched eggs to determine cause of death and observe abnormalities.

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## Overheating and Turner Failure

If the embryo is dead at 18+ days and you have residual albumen seen in the bottom of the egg then there was transport problem with albumen via the sero-amnion connection.



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## Effect of High Temperature



**Normal**

**Hot**

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## Typical of weak overheated chicks



Notice the urates which is a result of breaking down of muscle protein to make energy (glucose).

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**Scabby navels, black button and string**

When the navel does not close all the way and some of the yolk sac and yolk is left on the outside to dry out. May also see blood from the CAM. This blood will also dry.

Usually caused by **high heat** or **low humidity** (chick needs to get out of the shell **FAST**)

Chicks may be much weaker than others.



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Exposed brain – **overheating** during incubation



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Intestines – exterior to body – Indication of **overheating** of the embryo



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**Why Turn the Embryo?**

- Turning encourages the growth of the :
  - growth of the Area Vasculosa on the yolk sac membranes
  - The yolk membrane (vitelline membranes) will be replaced by the yolk sac membrane




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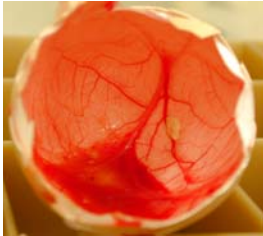
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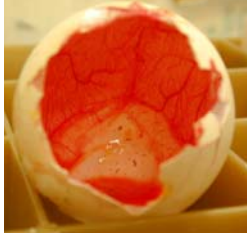


## Failure to turn causes

Normal CAM development



CAM not fully formed....impaired respiration surface



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## Why Turn the Embryo?

- Failure to turn also causes malpositions:
  - Head between the thighs
  - Head under left wing
  - Upside down
  - Head over wing

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## Turning Failures

- Failure to turn- common belief was that the embryo adhered to the shell membranes. Actually it influences the development of the allantois and yolk sac membranes.
  - Turning very important in the first 12 days when rapid development of extra embryonic components

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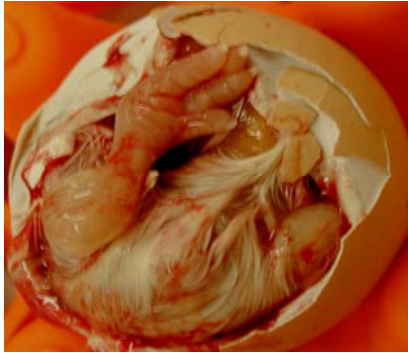
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### Overheating and Lack of Turning

Small end embryo (upside down)



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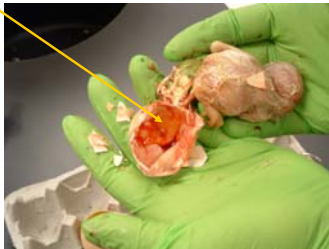
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### Temperature and Turning Malfunctions

- residual albumen



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### Albumen Transport to Amnion

- Absence of turning decreases amount of amniotic fluid (less albumen entering the amnion via the sero-amniotic connection)
  - Thus, by mid incubation the volume of albumen is greater in unturned than turned eggs – albumen should be less!!
  - **LESS NUTRIENTS AVAILABLE TO EMBRYO**

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### Humidity Problem Solving

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- Humidity too high slows hatch causes enlarged bellies
- Humidity too low you dry eggs and chicks (lose moisture from leg muscle) – chicks will have a difficult time standing.

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### Incorrect Moisture Loss due to Improper Humidity

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- Poor quality chicks (too much water or dehydrated)
- Residual albumen
- increased late dead
- increased navel buttons
- increased red hocks

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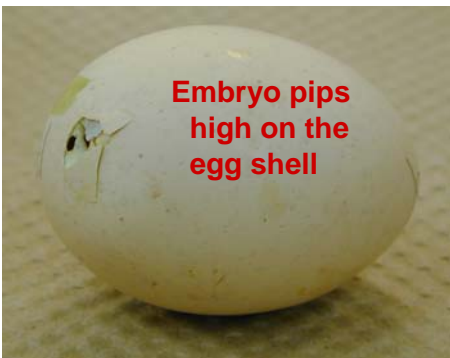
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Inadequate moisture loss resulting in small air cell



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## **LAB: Problem solving hatch**

- Problem solving hatch
  - Residue examination
  - Contamination
  - Examine chicks
- Book – Experiments in Poultry discussion

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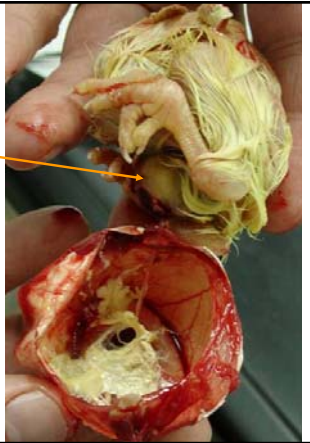
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Notice the urates, yolk not completely absorbed.

Generally seen in high incubation temperature, or turning problems causing malpositions.



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Not really a problem, but huge pipping muscle

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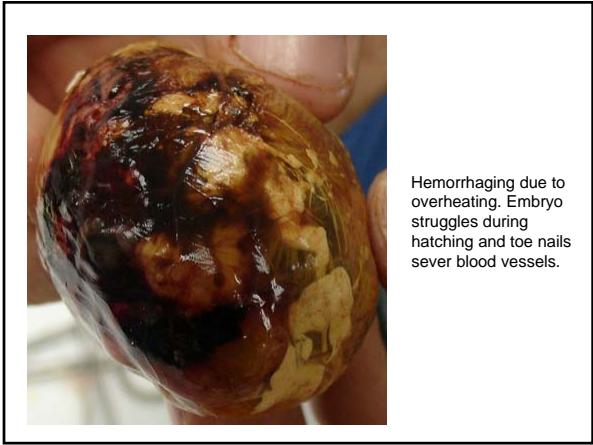
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**4H Curriculum Training**

- Experiments and understanding

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