An egg a day – the physiology of egg formation

Abstract
Modern hybrid laying hens can produce more than 300 eggs per year and more than 500 eggs in an extended life cycle. This would not be possible without continuous ovulation, a trait already observed in the ancestors of our modern layers. In the ovary of newly hatched hens more than 12,000 oocytes are present. Of these, only a small proportion will later acquire yolk and develop into mature ovulatory oocytes. With the onset of sexual maturity follicles with different sizes develop by accumulation of lipids resulting in a hierarchy of follicles which ensures the development of only one pre-ovulatory follicle per day. The precise regulation of follicle selection is only partially understood. It is assumed that the pituitary hormones FSH and LH play a dominant role in this process. In contrast, formation of the yellow yolk is well described. With the exception of immunoglobulins all proteins and lipids are synthesized in the liver and transferred from blood stream into the developing follicle. Yolk contains approximately 36% lipids, 17% protein (largely immunoglobulins) and only a minor fraction of free carbohydrates and inorganic components (<1%), the rest is water. Maternal antibodies in the yolk are exclusively of the IgY type. They are made by B-lymphocytes in the spleen and other lymphoid organs, secreted into the circulation and actively transported in the ovary into the yolk. Yolk formation is completed 24 hours before ovulation. After ovulation the follicle is placed in the infundibulum where the vitelline membranes are formed during the 1 hour passage. They separate the yolk from the album which is secreted by glandular and goblet cells of the magnum during the following 2-3 hours. 90% of the dry matter of the albumen is protein which is exclusively produced by the cells of the magnum. With the passage into the isthmus formation of the eggshell membranes starts which is followed by formation of the eggshell in the uterus. This process is controlled by the ovulation and several neuroendocrine factors which regulate the secretion of osteopontin and the expression of calcium transport proteins and calbindin. Within 11-15 hours 2.0-2.5 g of calcium are deposited in the eggshell. Two third of the daily calcium requirement is derived from intestinal resorption the remaining third is mobilized from unique bone structures called the medullary bone. This bone compartment is restored during the periods between eggshell formation. As in mammals, calcium homeostasis is regulated by 1,25-dihydroxyvitamin D3 and parathyroid hormone.

In recent years proteomics have been used intensively to unravel proteins involved in the formation of the different egg membranes, albumen and the eggshell and identified novel proteins protecting the egg and the embryo from pathogens. In addition, new transcriptomic data help to better understand the regulation of egg formation still only partially understood.

Keywords
chicken egg, production, egg structures, ovary, reproductive tract
Introduction
Modern hybrid laying hens can produce more than 300 eggs per year and more than 500 eggs in an extended life cycle. This would not be possible without continuous ovulation, a trait already inherited in the ancestors of our modern layers (1).

In the ovary of newly hatched hens more than 12,000 oocytes are present. Of these, only a small proportion (250-500) will later acquire yolk and develop into mature ovulatory follicles to give rise to egg formation (2). Here I will briefly describe the stepwise development of the main structures of the chicken egg in the ovary and during its passage through the reproductive tract.

Follicle maturation
With the onset of sexual maturity follicles with different sizes develop by accumulation of lipids resulting in a hierarchy which ensures the development of only one pre-ovulatory follicle per day. Sexually mature hens harbor numerous primordial follicles in the cortex of the ovary with up to 1 mm in size. This resting pool of follicles gives rise to a restricted number of primary follicles which further mature into so called pre-hierarchical follicles from which only one follicle (pre-ovulatory) is selected to terminally mature per day (3). The regulation of this selection process is only partially understood. It is assumed that inhibitory signals prevent the expression of the receptor for follicle stimulating hormone (FSH) which is secreted from the pituitary gland. Loss of this inhibitory signal in individual follicles leads to FSH-receptor expression and FSH-mediated synthesis of steroid hormones and factors supporting survival of follicle associated cells. Subsequently, the FSH effect diminishes and luteinizing hormone (LH; another pituitary hormone) regulates further follicle maturation and accumulation of yolk. Initially, primordial follicles are recruited to the cortical surface and eventually become connected to the blood supply and nerve system via the follicle stalk. Maturation from primordial follicles via primary follicles into pre-hierarchical follicles is associated with accumulation of white yolk rich in lipoproteins. These follicles are 6-8 mm in diameter and can easily been seen on the ovary surface. The terminal differentiation of such follicles to the pre-ovulatory follicle takes 4-6 days and is characterized by the massive accumulation of yolk (2g per day) a process completed 24 hours prior to ovulation (4).

Yolk composition
Yolk mostly consists of fat (36%), protein (17%) and water; 65-70% of the fat fraction are triacylglycerols, 25-31% phospholipids and 4-5% cholesterols. The protein fraction is composed of α-livetin (14%; = serum albumen), β-livetin (41%; = glycoproteins) and γ-livetin (45%; = IgY). Carbohydrates, vitamins and inorganic compounds represent less than 1% of the yolk. The characteristic color of the yolk is caused by carotenoids, in particular by xanthophylls (2). Both yolk fats and yolk proteins are synthesized in the liver a process induced by estrogen and testosterone secreted from maturing follicles in the ovary. The fat fraction is transported in the blood as very low density lipoproteins (VLDL). VLDLs bind to a specific receptors called LR8 which

Figure 1: The reproductive tract of the chicken, stepwise egg formation
is highly expressed on the oocyte membrane and are subsequently endocytosed into the yolk. This receptor has been studied in some detail. In addition to VLDL it transports proteins and protein associated vitamins. Mutations of the LR8 receptor have been observed in hens causing hyperlipidemia and a loss of fat transfer into the oocyte leading to infertility (5).

Immunoglobulin Y (IgY) is the only major yolk protein not being synthesis in the liver. It originates from plasma cells in lymphoid tissue which differentiate from B-lymphocytes during immune responses (6). IgY is actively transported from the blood into the oocyte a process mediated by a specific receptor in the oocyte membrane. The receptor is a member of the mannose receptor family and called FcRY (7). As a consequence of the receptor mediated transfer IgY composition in the yolk precisely reflects blood IgY composition. Up to 100 mg of IgY can be found in the yolk which provides maternal antibodies to the developing embryo and hatching. In addition, this IgY is a rich source of antibodies for diagnostics and therapy.

**Formation of the egg in the reproductive tract**

Rupture of the follicle wall leads to the release of the ovum which is captured by the infundibulum and thus funneled into the reproductive tract (Figure 1). Fertilization, formation of the vitelline membrane and of the first layer of albumen of the egg takes place in the infundibulum. The majority of albumen is produced during the passage through the magnum and subsequently egg shell membranes are formed in the isthmus. The final step of egg formation, the production of the egg shell, takes place in the uterus or shell gland before the egg is released by oviposition. This entire process takes 24-27 hours with most of the time required for shell formation. The structures of the egg are shown in figure 2. As discussed, 15-60 minutes after oviposition the next ovulation takes place.

**Infundibulum**

The ovum resides up to one hour in the infundibulum. During this time a membrane similar to the zona pellucida of mammals is formed which separates the ovum from the albumen. Recent studies have shown that at least 137 proteins are involved in the formation of the vitelline membrane most of them with unknown functions. However, it is known that it contains high concentrations of antimicrobial peptides (β-defensins, lysozyme, proteases), thus forming an effective inner barrier against infection (9).

**Magnum**

Subsequent to the infundibulum the ovum moves through the magnum where albumen formation proceeds during the next 3-4 hours. The main constituent of albumen is water with 88%. 90% of the dry matter are proteins, 6% are minerals and 3.5% is free glucose. In contrast to the yolk proteins, proteins of the albumen are
not produced in the liver but by the mu-
ocosa of the magnum. The tubular glands
secrete ovalbumin, ovotransferrin, ovo-
mucoid and lysozyme, while goblet cells
provide ovomucin and avidin. Synthesis
of these proteins is regulated by estrogen
and progesterone and continues even
in the absence of a forming egg. In con-
trast, release of the preformed proteins is
induced by mechanical stimulation as the
forming egg moves down the magnum
and is independent of endocrine or neu-
ronal regulation. Albumen is well known
for its antimicrobial properties caused by
numerous peptides including lysozyme,
avidin, cystatin and several defensins. At
the same time chalazae form a pro-
cess that starts early in the infundibulum.
They are made up of protein fibers which
connect the yolk and the shell membrane
and thus ensure the central position of the
yolk. Due to the rotation of the forming
egg during the passage down the mag-
num the fibers wind to form the characte-
ristic structure.

The albumen is not homogenous but forms
different areas of different viscosity with a more li-
quid inner egg white, the thick egg white
and chalazae and an outer more liquid lay-
er. This final structure develops as the albu-
men becomes increasingly hydrated in the
distal part of the magnum and the uterus a
process mediated by solute secretion (sodi-
um, calcium, magnesium and chloride) and
passive water movement.

**Isthmus**

After transfer into the isthmus formation
of the inner and outer shell membranes
by a mesh of protein fibers is initiated.
These membranes are closely spaced for
the most part of the egg but segregate
at the blunt end to form the air chamber.
While they are impermeable for egg white
they permit the exchange of water, gas (in
particular oxygen) and minerals. On their
outer surface projections form which are
called mammillary cores made up of pro-
teins, carbohydrates and mucopolysac-
charides. It is these structures where the
formation of the egg shell is initiated.

**Uterus (shell gland)**

With the arrival of the egg in the uterus
hydration of the egg white continues. Wa-
ter is pumped across the shell membranes
which lead to a volume increase and the
development of the characteristic ovoid
form. Consequently, the forming egg gets
in close contact with the uterine wall per-
mitting efficient formation of the egg shell.
This process takes up to 19 hours and is
characterized by the secretion of an orga-
nic matrix of glycoproteins and mucopo-
lysaccharides which becomes calcified to
from the egg shell (11). Calcification requi-
res approximately 2 to 2.5 g of calcium per
egg which forms calcite crystals, a highly
stable form of calcium carbonate (CaCO3).
The crystalline structures grow up from
the mammillary cores to form into high-
lly organized palisades giving the unique
strength to the egg shell. Layers producing
300 eggs per year require nearly 1.8 kg of
calcium matching their own body weight.
60-80% of the calcium is absorbed from
the intestine during shell formation if hens
are provided with the recommended 3.6-
4% of calcium in the feed. The remaining
20-40% of calcium required is mobilized
from the bone. Enteral resorption of calci-
um is regulated by 1,25-dihydroxyvitamin
D3 while mobilization of calcium from the
bone is under control of 1,25-dihydroxyvi-
tamin D3 and parathyroid hormone (PTH).
Calcium mobilized from the bone will be
replenished when the uterus is empty and
calcium absorbed in the gut is fully availa-
ble for bone matrix formation. Importantly,
hens develop a unique form of bone ma-
trix called the medullary bone. Two weeks
prior to lay a meshwork of interconnecting
spicules develops under the control by
estrogen and testosterone in the long bo-
nes. This structure accounts for up to 12%
of the total bone calcium but provides littl
support for bone stability. However, mo-
bilization of medullary bone is 10-times
faster than that of cortical bone and thus
can quickly make up for a lack of calcium
resorption from the gut (4).

To form the egg shell calcium and bicar-
bonate (HCO3-) must be secreted into the
uterine lumen to form the calcite crystals
(CaCO3). Calcium is taken up by epitheli-
al cells in the uterus from the blood by a
Ca2+-channel and transferred by a Ca2+
binding protein (calbindin 28K) to the api-
cal membrane where it is actively pumped
into the uterus lumen against a concen-
tration gradient. Several Ca2+ transporters
have been described and recent proteo-
ic studies have added new candidates
to the list (12).

To maintain a low Ca2+ concentration in
the epithelial cytoplasm which is critical
for cellular homeostasis Ca2+ is not only
bound to calbindin but also transported
into cellular storage compartments (en-
doplasmatic reticulum) from where it can
quickly be released during active shell
formation. HCO3- is generated from wa-
ter and carbon dioxide (CO2) in the cell
and transferred into the uterus lumen by
a transporter secreting HCO3- and taking
up Cl-in exchange. In the lumen Ca2+ and
HCO3- react to calcite crystals to build the
mineralized shell matrix.

The final step prior to oviposition is the de-
position of a waxy membrane on the egg
shell called the cuticula. It is composed of
polysaccharides, lipids and more than 50 proteins many of them with antimicrobial activities. Accordingly, its primary function is to prevent penetration of microbes into the egg and to limit the loss of water from the egg (13).

**Oviposition**

This process is initiated by the relaxation of the uterine sphincter and the contraction of uterine smooth muscles. It is regulated by neurohypophysial hormones, primarily oxytocin and arginine-vasotocin as well as prostaglandins (e.g. PGF2α) synthesized locally within the reproductive tract. Within the next 60 minutes after oviposition the next ovulation will take place.

**Conclusion**

Egg morphology, composition and formation have been studied intensively for more than a century and a large body of literature is available. However, many of the regulatory circuits are only partially understood. New technologies such as next generation sequencing and proteomics (12, 14, 15) may help to gain new insights into the multiple steps of egg formation and the precise composition of yolk, albumen, egg membranes and the egg shell. Reverse genetics are also progressing in the field of avian biology and should finally enable functional in vivo studies of selected genes (13).

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