12.1 INTRODUCTION

« Les Cerastes comme aussi les vipers en toutes parts, rendent leurs petits en vie, comme aussi fait la Salamandre » (Belon 1554, p. 122).

The above quotation (“Cerastes as vipers in general give birth to living young like the Salamander”) is one of the earliest, if not the earliest, written evidence that a urodele (*Salamandra*) is viviparous. Belon (*l.c.*) obviously assumed knowledge of viviparity in *Salamandra*. Viviparity of vipers was described already in the fourth century B.C. by the Greek philosopher Aristotle (Peck 1970).

It is not the aim of the present overview to summarize the history of “viviparity” in salamanders (for further readings see Francis 1934; Greven and Thiesmeier 1994; Greven 2002). However, the definition of viviparity and the question whether ovoviviparity (often defined as birth of aquatic larvae), (true) viviparity (birth of metamorphosed juveniles) or, when emphasizing (the hard to recognize) trophic relationships as crucial, lecithotrophy (embryos are exclusively yolk dependent) and matrotrophy (young are supplied with additional maternal nutrition) occur among salamanders always has been a matter of debate (*e.g.*. Salthe and Mecham 1974; Wake 1982; 1993; Thiesmeier and Haker 1990; Greven and Thiesmeier 1994; Blackburn 1995; Greven 1998).

A generally accepted definition of viviparity does not exist. Use of the term viviparity in its literal meaning, *i.e.*, the birth of *live* young, implies that oviparity, *i.e.*, egg laying, used commonly as the converse of viviparity, is (though perhaps oversubtely argued) the birth of *dead* offspring (see the note in Packard 1989). In the following I make, therefore, the state of the born progeny the basis of terminology as suggested previously (see Greven 2002) and distinguish among urodeles: oviparous species that deposit eggs fertilized
outside the genital tract as in externally fertilizers or when passing through the cloaca; larviparous species that release true larvae; and pueriparous species that give birth to transformed young (derived from the Latin word *puer* = child, boy, adolescent).

Data about the reproductive biology of the urodele species that have developed larvi- and pueriparity are widely scattered in the literature. Many valuable observations have been largely ignored on account of their regional character, the language in which they are published, or their age (reviewed partly by Greven 1998, 2002; Greven and Guex 1994; Guex and Greven 1994). Nevertheless, knowledge of the reproductive biology of larvi- and pueriparous species is often sketchy. These species also are often protected by law in the countries in which they occur, hindering further research. Thus, various aspects of their reproductive biology are often a matter of speculation.

Reproductive modes affect the whole life history of an animal. However, the following account focuses on structural and physiological specializations for internal development in salamanders and does not deal with ecological implications (for further readings see the reviews mentioned and Bruce, chapter 13 of this volume).

### 12.2 OCCURRENCE OF LARVIPARITY AND PUIERIPARITY AMONG URODELES

Apart from a single, but controversial exception of facultative larviparity (see below), only the genera *Salamandra* and *Mertensiella*, belonging to the “true” salamanders, have achieved obligate larvi- and pueriparity. Young develop in the modified posterior-most region of the oviduct, the uterus (see Greven, chapter 5). These reproductive modes enable the animals to lead terrestrial lives to a large degree. Only larviparous females must return temporarily into the water to deposit their offspring.

The genus *Salamandra* that occurs in Europe, North Africa and the Near East consists of the fire salamander, *S. salamandra* complex, and two species of Alpine Salamanders, *S. atra* and *S. lanzai*. The Fire Salamander complex currently includes four species each with its associated subspecies (for details see Steinfartz *et al.* 2000). Structural and physiological data are known for only a few taxa. *Salamandra* species and/or subspecies give birth variably to larvae as well as transformed young. Among others, larviparity is known from *S. salamandra salamandra* and *S. salamandra terrestris*. Most studies have been made on these taxa that both will be called simply *S. salamandra* in portions of the following. Also *S. infraimmaculata* and *S. salamandra gallaica* are larviparous, whereas *S. salamandra fastuosa* has a mixed reproductive strategy, i.e., they give birth variously to larvae and metamorphosed juveniles. *Salamandra salamandra bernardezi*, however, only bears fully metamorphosed young (for further readings see Greven and Thiesmeier 1994). Both species of the Alpine Salamander (see above) are always pueriparous.

The genus *Mertensiella* includes the oviparous *Mertensiella caucasica* and the *Mertensiella luschani* complex distributed in southwestern Turkey and
the adjacent Aegean islands. As far as known, all taxa in this complex are pueriparous (e.g., Özeti 1979; Guex 1994; for further references, see Greven and Thiesmeier 1994). On the basis of mitochondrial DNA sequences, *Mertensiella luschani* was recently recommended to be placed in the genus *Salamandra* (Weisrock et al. 2001).

Larviparity of the usually oviparous paedomorphic olm, *Proteus anguinus* (Proteidae), was first recorded in 1831 by Michahellis (fide Briegleb 1962) and was subsequently noticed occasionally in freshly captured specimens or in specimens kept inadequately (see Briegleb 1962), but these observations were not included in recent reviews (Parzefall et al. 1999). *Proteus anguinus* always produces eggs when properly maintained in the laboratory, and therefore oviparity is assumed to be the normal reproductive mode (Briegleb 1962). The multiple and independent records of larviparity, however, may indicate the plasticity of this species to retain eggs for their development in the oviduct or different reproductive strategies among populations.

The evolution of larviparity and pueriparity among salamanders appears largely to be determined by heterochronous processes. Although not fully documented as yet, heterochronisms can be recognized comparing the development of oviparous, larviparous and pueriparous species. Most obvious are for example: (1) the different timing of ovulation (stepwise, e.g., in the oviparous *Triturus* ssp., at once in larvi- and pueriparous salamanders); (2) the timing of hatching (*Salamandra atra* hatches at an earlier developmental stage than *Salamandra salamandra* and *Triturus* ssp.); (3) earlier onset of feeding in *S. atra*; (4) forced hatching of earlier developmental stages by hatching enzymes of more advanced siblings as in *S. salamandra fastuosa* and *S. salamandra bernardezi* facilitating adelphophagy; (5) prolonged time of metamorphosis in *S. atra*; and (6) a general reduction of larval traits during intrauterine development (discussed in greater detail by Guex and Greven 1994; see also Dopazo and Alberch 1994; Alcobendas et al. 1996).

Larviparity and pueriparity evolved from oviparity, but selective pressures favoring this evolution have not been satisfactorily discussed in the literature. Most authors argue – more or less plausibly at first glance – that these reproductive strategies evolved as a consequence of selective pressures tending to reduce the aquatic larval period. Various factors that may elicit this evolution and may act in combination have been considered, e.g., predation of the larvae, sweeping away of the larvae by the water current (that favors development of larger larvae), deficiency of planktonic food for small larvae, absence of breeding waters in dry habitats, and short seasons for growth due to cold temperatures as in high mountains (Joly 1968; Häfeli 1971; Fachbach 1976a,b; Thiesmeier and Haker 1990; Joly et al. 1994; Dopazo and Alberch 1994). The mixed reproductive strategy (larviparity and pueriparity) of *Salamandra salamandra fastuosa* was suggested to be an adaptation to the harsh conditions of high altitude environments (Joly 1968; Gasser and Joly 1972; Fachbach 1969, 1976a,b), but populations of this species are also found in the lowland suggesting that such factors may differ even within a given species (Dopazo and Alberch 1994).
However, selective pressures that favored evolution of larvi- and pueriparity – perhaps the necessity to reduce risks for the female, e.g., during the deposition of eggs, etc. – are very likely not the same that favor recent distribution of such species. Once established, larviparity and pueriparity or at least prolonged egg retention may be favored by the different factors mentioned.

Further, reproductive strategies are costly, but the different costs, such as mechanical, physiological, genetic, immunological and nutritional ones, have not been estimated for larvi- and pueriparous salamanders as yet.

The question whether larvi-and/or pueriparity evolved once or multiply in Salamandra has not been satisfactorily answered (e.g., Alcobendas et al. 1996; Veith et al. 1998).

12.3 INSEMINATION AND FERTILIZATION

About 90% of extant urodeles practice internal insemination after uptake of spermophores preceded by a more or less complex courtship (see Sever, chapter 9; Houck and Arnold, chapter 10 of this volume). Sperm then penetrate the jelly layers when eggs pass the spermathecae, which are situated in the roof of the cloaca. Thus, fertilization takes place internally, but beyond the oviduct. Courtship and mating of the known larvi-and pueriparous salamanders takes place on land (see Houck and Arnold, chapter 10). Interestingly, previous authors have recorded rather incidentally gathering of males and females of Salamandra atra, in spring during the day or after rain, and a kind of mass mating (Harms, 1946, refers to older literature; Vilter et al. 1959, some details are dubious in this paper). The observation that numerous males of S. salamandra are apparently “waiting” for females on rainy nights (Th. Mutz and G. Clemen, personal communication) suggests a similar phenomenon in this species.

However, when the offspring develops in the oviduct, oviductal fertilization is a prerequisite. The shift of fertilization into the oviduct was suggested as the crucial step in the evolution of larvi- and pueriparity in salamanders (von Wahlert 1953).

Early findings report on cleaving eggs in the oviduct in Salamandra salamandra (e.g., Schwalbe 1896; reviewed by Guex and Greven 1994), and the presence of different developmental stages along the oviduct, with more advanced stages toward the cloaca, confirms fertilization in the upper subdivisions (Zakrzewski 1976). Joly and Boisseau (1973) detected spermatozoa in the posterior and middle subdivision of the glandular pars convoluta of ovulating S. salamandra. Spermatozoa not used for fertilization were resorbed by the gland cells within 48-72 hrs. Zakrzewski (1976) studied the reproductive cycle of S. salamandra in Poland and found small numbers of spermatozoa in the oviduct of pregnant females in April after hibernation. In May, a high number of motile spermatozoa occurs throughout the length of the oviduct in females starting ovulation, and a small number remain in July when the embryonic development starts. It was speculated that spermatozoa arose from
spermatophores transferred in the preceding year, but long sperm storage in
the spermathecae of *S. salamandra* has been questioned recently (see Sever,
chapter 9 of this volume). However, Guex (cited in Guex and Greven 1994)
reportedly isolated living sperm from a female *S. atra* kept for more than two
years without a male.

Structure and nature of egg-jelly at the time and at the site of fertilization
(see Greven, chapter 5; Watanabe and Onitake, chapter 11 of this volume) are
unknown in larvi- and pueriparous forms. The jelly layers of eggs fertilized in
the anterior oviduct in *Salamandra salamandra* (see Joly and Boisseau 1973) may
be incomplete. Lostonlen et al. (1976) illustrated a two-layered jelly (“capsule
larvaire”) surrounding the intrauterine larva of this species and assumed
solubilization of the innermost layer.

The oviduct of *Salamandra atra* has fewer gland cells. The amount of jelly is
not sufficient to encase all developing eggs (Vilter 1967, 1968; Vilter and Vilter
1962), which are also smaller (1.0 to 3.0 mm in diameter; see Fig. 12.5A) than
those of *S. salamandra* (that may reach 5 mm in diameter; Wunderer 1910;
Häfeli 1971). Therefore, only the “embryonic” egg, usually one per uterus, is
provided with a complete jelly and will be fertilized. Interestingly, the oviduct
produces considerably more jelly when artificially stimulated by estradiol benzoate (Vilter and Vilter 1962; Guex and Greven 1994).

Embryonic eggs are coated by a tough egg-jelly nearly twice or more as
thick as the diameter of the egg (Wunderer 1909, 1910; Vilter 1967; Vilter
and Vilter 1967) that may be due, however, at least partly to swelling. It
was suggested that fertilization takes place when the thick egg-jelly of the
embryonic egg in the uterus protrudes into the cloacal chamber and brings the
uterine mouth close to the spermathecae. The thick jelly layer of the embryonic
egg takes up a considerable amount of fluid during early development (see
Fig. 12.5C; Häfeli 1971; Guex and Greven 1994). The mode of fertilization seen
in *S. atra* may be considered as a secondary shift of the fertilization site back to
the cloaca or splitting of species occurred earlier.

The remaining 20 to 104 (!) eggs per uterus are either encased incompletely
or lack any jelly. They do not become polyspermic as previously suggested
and attempts to inseminate them have not been successful (Häfeli 1971; Guex
and Greven 1994). In the uterus, they disintegrate, forming a creamy mush that
will be eaten by the young (see Fig. 12.5D; 12.5.5).

### 12.4 OVIDUCT AND UTERUS

The oviduct and uterus have been described in chapter 5. Here the most
conspicuous differences among the oviducts of larviparous, pueriparous,
and oviparous species will be mentioned.

Five main parts are distinguishable in the oviduct of *Salamandra salamandra*:
the *pars recta*, three glandular subdivisions of the *pars convoluta*, and the uterus
(Fig. 12.1; Greven 1977, 1980a,b, 1981; 1998, 2002; Greven and Rüetborries
1984; Greven and Baldus 1984; Greven and Guex 1994). This zonation holds
also for *Mertensiella luschani* (Polymeni and Greven 1992) and *S. atra* (Greven 1998; Guex and Greven 1994), and a similar organization is expected for the remaining species and subspecies not examined to date. In *S. atra*, Vilter (1967) and Niederl (1973) described a fourth glandular subdivision with glandular cells slightly different from those of the previous one. In *S. salamandra* and *M. luschani*, tubular glands occur in the oviduct, whereas in *S. atra*, a simple columnar glandular epithelium is predominant. Smaller gland cells occur in both species (Fig. 12.1; see Greven, chapter 5).

Generally, the larvi- and pueriparous forms examined so far tend to reduce the posterior subdivision of the glandular *pars convoluta* and to secrete more acid than neutral mucopolysaccharides (Vilter 1967, 1968; Boisseau 1980; Greven 1980a, 1998, 2002). As the mucopolysaccharides form the egg-jelly layers, these changes should have some consequences for their composition and strength. Unfortunately details are lacking.

Compared with oviparous species, the *pars convoluta* in larvi- and pueriparous forms appears less convoluted and is reduced in length in favor of the uterus. The latter is the most obvious modification of the oviduct.

The histology of the uterus in *Salamandra salamandra* and *S. atra* has been known since the late 19th century (Wiedersheim 1890; Schwalbe 1896; see also Francis 1934). It is an aglandular portion of the *pars convoluta* exhibiting numerous smooth muscle cells and a thick connective tissue layer in non-pregnant females (see Greven, chapter 5). Vascularization is rich in both pregnant and non-pregnant females (Fig. 12.2A,B). Most of the capillaries are situated immediately under the simple cuboidal or often very thin epithelium (Fig. 12.2B,C) that is largely smooth and lacks ciliated cells (Fig. 12.2D). Epithelial cells are moderately secretory (Fig. 12.2E) and discharge sulphated glycoproteins, possibly mediated by hormonal and mechanical stimuli (Greven and Robenek 1982), that contribute to the uterine fluid (Lostanlen *et al.* 1976; Greven 1977). Secretion seems to be asynchronous, i.e., cells behave more or less individually as indicated by the absence of gap junctions between adjacent cells (Greven and Robenek 1980a). Activity of several hydrolases and oxidoreductases has been demonstrated in epithelial cells; occurrence of the latter indicates the strong oxidative metabolism of the tissue (Greven *et al.* 1986).

Based upon a series of investigations, mainly on pregnant females of *Salamantra salamandra*, the uterine epithelium (as most epithelia) is a typical absorbing epithelium. Evidence that cells are involved in the transport of ions and solutes toward the vascularized connective tissue (Fig. 12.3) was deduced from: (1) their ultrastructure, namely the presence of numerous mitochondria and long, partly distended intercellular spaces (Fig. 12.2E) that are sealed apically by flexible *zonulae occludentes* and open basally toward the subepithelial tissue (Greven 1977, 1980a, 1998; Greven and Robenek 1980a); (2) indirect evidence of an asymmetrical (i.e., basolateral) Na\(^+\)-K\(^+\)-ATPase activity; (3) precipitation of chloride and cations (among others, sodium) in the intercellular spaces, known as the main route of ion and solute transport;
Fig. 12.1  Diagram of the oviduct of *Salamandra salamandra* with the different subdivisions and the relative frequency of cell types that form the epithelium. From Greven, H. 1998. Journal of Experimental Zoology 282: 507-525, Fig. 4.
and (4) measurement of a trans-uterine potential of 15 to 25 mV (the inside of the uterus is negative) and a circuit current of 200-300 µA produced by actively transported ions, both of which decrease after inhibition of the Na⁺-K⁺-ATPase with ouabain (Greven 1980b). Changes of these parameters should be expected during the reproductive cycle and the seasons, but no further information is available. Presence of this membrane-bound pump may create a suitable environment for the development of the offspring (see 12.5.3). Such mechanisms are ubiquitous in vertebrate epithelia and surely exist in the uteri of the species not investigated.

Thinning of the uterine wall during pregnancy in Salamandra salamandra is caused largely by the mechanical stress exerted by the growing offspring (Lostanlen et al. 1976; Greven 1977). Zonulae occludentes described from the uterus of pregnant and non-pregnant S. salamandra obviously can adapt to changes in this stress (Greven and Robenek 1980a). Structural similarities between the pregnant and non-pregnant state (Lostanlen et al. 1976; Greven 1977; Greven and Robenek 1980b) indicate that the uterine epithelium of S. salamandra has only basic functions common to almost all epithelial tissue (Greven and Robenek 1980b). After ovariectomy, the epithelium seems disordered and coherence of capillaries and the epithelium is weakened (Lostanlen et al. 1976).

Uterine changes in the course of pregnancy are more conspicuous in the pueriparous Salamandra atra (Vilter and Vilter 1962; Niederl 1981; Guex and Greven 1994), but flattened and cuboidal epithelial cells occur in both pregnant and non-pregnant females (Fig. 12.4A,B). When larvae have reached Schwalbe stage I (see 12.5.5), cell and nuclear volume of epithelial cells increase continuously, indicating altered metabolic activity of the uterus. More prominent cyclic changes, however, occur in a specialized area of the uterus, the zona trophica (see 12.5.5).

12.5 MOTHER-OFFSPRING RELATIONSHIPS

The length of gestation varies from a few months to some years depending on the species and climatic conditions. Gestation in Salamandra salamandra has been reported or estimated to be five to 14 months; in S. atra and S. lanzai, up to four years; in S. atra aurorae, up to three years; and in Mertensiella luschani,
Fig. 12.3  Suggested origin and transport of solutes (water, ions, etc.) in the uterus of pregnant *Salamandra salamandra*. Black arrows: active transport; clear arrow: passive transport. Sodium enters the cell passively, moves down the ion’s electrochemical gradient across the lumenal cell membrane and is pumped subsequently from the cellular compartment into the intercellular spaces. The entry of sodium creates a local increase in osmotic pressure which draws water and solutes from the lumen and the cells into this region and toward the subepithelial tissue. Larvae also have to take up solutes and water. From Greven, H. 1998. Journal of Experimental Zoology 282: 507-525, Fig. 8.
up to a little more than one year (Joly 1968; Joly and Picheral 1972; Özeti 1979; Guex 1994; Miaud et al. 2001). Maternal-embryonic interactions during this time involve respiratory, osmoregulatory, endocrinological, immunological, and trophic relationships.

There is evidence that intrauterine larvae of *Salamandra salamandra* stop or at least slow down growth (judged by the ossification of the skeleton; Amend and Greven 1996) in winter-time until spring when most parturitions take place (see also Gasche 1939). Continuous growth of the often numerous larvae during hibernation could cause some problems regarding the space and the limited yolk supply. Tubular bones in intrauterine *S. atra* show development throughout the intrauterine period (Fachbach 1988). Lines of arrested growth, however, occur in the humerus of intrauterine larvae of *S. lanzai*, which results in an estimation of the gestation period of up to four years (Miaud et al. 2001).

In *Salamandra salamandra*, a central role of the thyroid gland is suggested. The thyroid gland of intrauterine larvae is relatively active just before and at the beginning of hibernation. Thus, it is speculated that a relative high thyroxin concentration could keep low the level of the somatotropic hormone prolactin.
and, consequently, could inhibit growth. Thyroid hormones also increase activity of the urea cycle, necessary for ureotelism (see 12.5.3) achieved by the intrauterine larvae (Schindelmeiser 1985). Unfortunately, details and comparable studies on other larvi- or pueriparous species do not exist.

Intrauterine larvae of Salamandra atra and S. salamandra and even larvae of the oviparous Triturus alpestris are able to control surface tension by substances that appear to be secreted by the skin. Only small amounts of fluid are necessary to keep the embryo covered with the uterine fluid. Substances from the epidermis have been supposed to serve also as signals for the female to recognize pregnancy or readiness for birth (Guex and Greven 1994).

12.5.1 Endocrinology

There are only a few investigations regarding the endocrinology of larvi- and pueriparity. As in other urodeles, oocyte maturation coincides with highly active glands in the oviduct (Joly and Picheral 1972; Zakrzewski 1976). Concentrations of steroids during the reproductive cycle of female Salamandra salamandra terrestris and S. infraimmaculata (with an annual cycle) and S. salamandra fastuosa (with a biennial cycle at higher elevations) have been measured in the ovary and the serum of females. Levels increase after ovulation to the end of vitellogenesis, and gonadotropic cells of the pituitary are active during vitellogenesis (Garnier et al. 1986; Joly et al. 1994; Degani et al. 1997). Administration of estrogen provokes hypertrophy of the secreting oviduct of S. atra (Vilter and Vilter 1962; Guex and Greven 1994).

However, understanding of the endocrine control of reproduction is very limited. As judged by histology (Salamandra atra; Harms 1946; Vilter and Vilter 1960, 1964) as well as histochemistry and ultrastructure (S. salamandra, Joly and Picheral 1972), corpora lutea are present during the gestation period. In pregnant females of S. atra, they are present in large numbers and persist for the first two years of pregnancy, but gradually became reduced towards the term of parturition. They may control oogenesis and activity of oviductal glands and stage I and stage II features of the uterus. The sharp decline of the number of corpora lutea coincides roughly with the onset of oocyte growth and the formation of the zona trophica at the transition of stage II to stage III (for staging see 12.5.5; Häfeli 1971; Niederl 1981; Guex and Greven 1994). Corpora lutea of S. salamandra show steroidogenic activity and steroid metabolism, but their exact role is unclear as embryos remain alive and develop after ovariectomy. In ovariectomized pregnant animals, the epithelium seems to be disordered, and the uterus is less supplied with blood (Joly and Picheral 1972; Lostanlen et al. 1976). However, the ultrastructure of the epithelial cells is not extraordinarily affected by any stage of pregnancy.

Neurohypophysial hormones evoke strong contractions of the oviducts of Salamandra salamandra and Salamandra atra. Experiments on isolated oviducts of non-pregnant females show strong reactions to vasotocin and extracts of the animal’s own pituitary gland. Reactions to arginine vasopressin and oxytocin were less intense. Pregnant females of S. salamandra injected with
high doses of vasotocin intramuscularly gave birth to their larvae (Heller et al. 1970). This was difficult to achieve in *S. atra* females at stage III. Perhaps a signal is needed from the intrauterine offspring about its developmental status, which permits the uterus to react. However, in *S. salamandra* birth of larvae extends over several days and in *S. atra* a few weeks may pass between parturition of the two young (Greven and Guex 1994; see 12.5.6). Changes of sensitivity dependent on the stage of the reproductive cycle and seasons have not been investigated. Previous studies by Gasche (1942), who induced premature birth in *S. salamandra* with several hypophysial hormones and external stress (light, temperature, etc.), suggest a greater sensitivity toward the spring and even differences of sensitivity among populations.

### 12.5.2 Respiration and Gas Exchange

Information on respiration and gas exchange is deduced from anatomical and histological studies. The rich vascularization of the uterus is conspicuous. Length of the network of blood vessels in the uterus of a pregnant, mediumsized female *Salamandra salamandra* was calculated to be 38 m (Greven and Guex 1994; Fig. 12.2A). There is no evidence, however, that the length or density of the network increases during pregnancy. Possibly, blood pressure increases and causes dilatation of vessels (Kaufman 1913; Greven 1998; Greven and Guex 1994). Thinness of the epithelium (in some areas < 3 μm) and the underlying capillaries reduce diffusion passage-ways considerably. Larvae of salamanders are equipped with long filamentous gills that are provided with numerous ciliated cells (Greven 1980c; Guex and Greven 1994). Cilia keep the intrauterine fluid in motion. Gills reach their maximal relative length in *S. atra* at the end of stage II (see 12.5.5). Gills surely absorb oxygen from the highly vascularized uterus either via the egg-jelly that encases the embryos of many larviparous species or directly, when the larva is hatched. In addition, the surface epithelium also may be regarded as a respiratory surface.

To my knowledge, physiological studies on respiration and gas exchange do not exist, but differences in embryonic and maternal blood oxygen affinity can be expected.

### 12.5.3 Osmoregulation and Excretion

Intrauterine development also poses problems with osmoregulation and the removal of metabolic wastes from the uterine environment.

Ion content (measurements of sodium only) of the uterine fluid of *Salamandra salamandra* correspond to that of the blood during pregnancy (approximately 80 mmol/l), whereas it is considerably higher (ca. 290 mmol/l) in non-pregnant animals (Greven 1998; Greven and Guex 1994). This indicates a maternally controlled uterine environment, but changes in the course of pregnancy are unknown. Also, larvae are participants of the uterine fluid to some extent, but again investigations on this subject are lacking. The epidermis and the thin gill epithelium of intrauterine larvae of *S. salamandra* show, as expected,
chloride in the intercellular spaces and Na\(^+\)-K\(^+\)-ATPase activity (Lewinson et al. 1987a; Greven and Guex 1994). Gills (Greven 1980c) and epidermis (Lewinson et al. 1982, 1984) possess two types of mitochondria-rich cells that reach the surface of the epithelium. They were more intensely investigated only after birth and show carboanhydrase-activity that increases during larval development. High Na\(^+\)-concentrations inhibit this activity. Mitochondria-rich cells have been suggested to be involved in gas exchange and acid-base regulation (Lewinson et al. 1982, 1984, 1987b). A study of Restani and Pederzoli (1997) on epidermal mitochondria-rich cells in newborn S. salamandra revealed expression of adhesion molecules and glycoproteins. These molecules perhaps maintain the architecture of skin components. No further studies are available.

Metabolic wastes have to be stored or to be removed via the blood vessels in the uterine connective tissue. Intrauterine larvae of Salamandra salamandra are sensitive to ammonia, but can tolerate concentrations of urea that exceed those in the uterus. Content of urea nitrogen in the blood plasma and uterine fluid of pregnant females is higher than in non-pregnant females, suggesting ureotelism of larvae. Activity of the hepatic ornithine-urea cycle (measured as activity of hepatic arginase) is known. When released in water, larvae return only partly to ammonotelism (Schindelmeiser and Greven 1981; Schindelmeiser et al. 1983). In S. atra, increasing urea amounts also have been demonstrated in the uterine fluid during pregnancy (Guex and Greven 1994). The same can be predicted for the other species, because of the restricted amount of fluid within the uterus.

12.5.4 Immunology

The intrauterine embryo as well as spermatozoa in the spermatheca and oviduct (Sever 1992) can be regarded as an allograft in their maternal host. Allograft rejection and immune reactions have been shown only toward the intrauterine offspring in Salamandra salamandra. In a series of experiments with cells in culture, Badet (1984), Badet et al. (1980), and Chateaureynaud et al. (1979) demonstrated that only the serum of pregnant females inhibits a cytotoxic reaction of spleen cells, also of pregnant females, against larval cells. One fraction of the serum specifically protects embryonic epithelial cells; a second one, however, is unspecific. An IgM and an alpha-2-macroglobulin, the latter linked with immunosuppressive properties only during pregnancy, is involved in this process. The protective effect appears to be enhanced with increasing number of embryos in the uterus, and cytotoxicity and protection is largely specific for the female’s own embryos.

12.5.5 Nutrition

In larvi- and puerciparous salamanders, the nutritive support of the young during development ranges from the initial yolk stores (lecithotrophy) to oophagy and adelphophagy, both of which are forms of embryonic cannibalism, and epitheliophagy. To facilitate nutrient transfer, some adaptations have
evolved, probably independently in part. With the exception of Salamandra atra, maternal tissue appears not to be specialized for nutrient transfer but precocial hatching to ingest food becomes important in some taxa.

Larvae of Salamandra salamandra and some other species and subspecies are arranged like a roll of coins within the uterus and are encased in jelly-layers to full term. Occasionally, unfertilized, but intact eggs can be found between the developing offspring, but these eggs do not serve as food (e.g. Kaufman 1913; Gasche 1939). Nutrients other than yolk have to penetrate the egg envelope. Larvae of S. salamandra take up amino acids that crossed the egg-jelly, however, in negligible amounts (Lostanlen et al. 1976), and amino acids reach the uterine lumen in S. atra during stage II and III (Guex and Greven 1994). Consequently, dry weight decreases during the gestation period (Kaufman 1913; Gasche 1939; Joly 1968; Greven and Guex 1994). Larvae can be removed from the uterus very early in development and reared in water (Gasche 1939).

In other species and subspecies, nutrients additional to the yolk are supplied during development and that requires hatching within the uterus. Salamandra salamandra bernardezi from Oviedo (Spain) gives birth to a few fully metamorphosed, relatively large young (Wolterstorff 1928). Developmental rates are, however, heterogeneous. Intrauterine larvae feed on degraded, unfertilized eggs (oophagy), that form an embryotrophe, and on less developed siblings (adelphophagy) (Bas and Gasser 1994; Guex 1994; Dopazo and Alberch 1994; Thiesmeier et al. 1994). Dopazo and Alberch (1994) described variation in the pattern of pueriparity in this species: (1) oophagy and adelphophagy, facilitated by a great number of unfertilized eggs and heterogeneous development, produced large metamorphosed young, as well as (2) a considerable reduction of clutch size and higher fertilization rate; lack of intrauterine feeding produced smaller transformed juveniles (see also Alcobendas et al. 1996).

The behavior of Salamandra salamandra fastuosa is more plastic. The number of fertilized and unfertilized eggs is variable as well as the size and weight and the developmental stage of larvae. Degrading unfertilized eggs and smaller siblings are cannibalized, and the same female can bear facultatively numerous larvae and/or fully metamorphosed young (Joly 1968; Gasser and Joly 1972; Dopazo and Alberch 1994). Oophagy and adelphophagy lead to strong intrauterine selection.

Mertensiella luschani helverseni normally gives birth to two fully metamorphosed young. In the uterus they feed on disintegrated eggs. Very likely, a zona trophica does not exist (Guex 1994).

With respect to pueriparous salamanders most data are available from Salamandra atra (among others, Czermak 1843; Wiedersheim 1890; Schwalbe 1896; Fachbach 1969; Háfeli 1971; Guex and Chen 1986; review of the older literature in Guex and Greven 1994). Young, usually one per uterus, feed on the yolk when still surrounded by the egg-jelly (lecithotrophic phase; Schwalbe stage I; Fig. 12.5C). Once they have left the jelly (Schwalbe stage II), they feed
on disintegrating eggs (Fig. 12.5D). During transition from stage II to stage III, the position of the larvae within the uterus is fixed; metamorphosis begins (Fig. 12.5E). In stage III, the embryotrophic eggs are consumed and the young measure 4-5 cm in length (Fig. 12.5F). Stage III larvae can be removed from the uterus and reared in water.

Gestation cycles in *Salamandra atra* last up to 4 years, depending on climatic conditions that are related to the altitude at which individuals are found (see Fig. 12.8; e.g., Wunderer 1909; Vilter and Vilter 1960, 1964; Häfeli 1971; Guex and Greven 1994). Secretion from the uterine epithelium (previously called “uterine milk”) starts with Schwalbe stage II and was assumed to be the essential source for nutrition (Vilter and Vilter 1964; Niederl 1981). There is no evidence for uptake of particulate nutritive substances across the gills as supposed previously (Schwalbe 1896; Häfeli 1971). Uptake of low molecular weight substances across the gills or the surface epithelium, although suspected, has never been proven. Of greater importance is a proliferating zone at the anterior end of the uterus termed the *zona trophica* by Fachbach (1969) but initially described in 1890 (Wiedersheim 1890; see Guex and Greven 1994). Cyclic changes were intensely studied by Guex and Chen (1986) and reviewed by Guex and Greven (1994) and Greven (1998). In this zone, mitotic activity is seen (Fig. 12.6 A), and large epithelial cells bulge into the uterine lumen and become detached from the underlying connective tissue, probably by apoptotic processes and/or necroses (Fig. 12.6B,C). The young ingest cells floating in the uterine lumen. When positioned with their head to the nutritive zone, embryos may scrape off cells from this area with the help of special toothed areas (“Zahnfelder”) of the upper and lower jaw (Greven 1984; Guex and Chen 1986; Fig. 12.5G). Often more than the half area of the *zona trophica* is free of epithelial cells independent of the position of the larvae and even blood vessels will be opened (Fig. 12.6B,C,E). Surprisingly, cells of the trophic zone do not accumulate glycogen or lipids in considerable amounts (Fig. 12.6C).

The *zona trophica* forms only in presence of embryos of the Schwalbe stage III, and its formation cannot be induced by progesterone and/or estrogen. Thus, it develops probably independent from maternal hormones; induction

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**Fig. 12.5** Pregnancy stages (according to Schwalbe 1896) and position of young in the uterus of *Salamandra atra* (A–C, E, F) and *Salamandra atra aurorae* (D). A. Female near ovulation. B. The embryonic egg enters the uterus. C. Larvae a short time before hatching, stage I. Note the swollen jelly envelope. D. Larvae at early stage II within the creamy mass of embryonic eggs. E. Transition from stage II to stage III. Note twins at the right side and the whitish zone (arrows) of the developing *zona trophica*. The head of the larva at the left side is directed to the cloaca, that of one larva at the right side to the *zona trophica*. F. Stage III larvae. From Guex, G.-D. and Greven, H. 1994. Pp. 161-208. In H. Greven and B. Thiesmeier (eds), Mertensiella Supplement 4, DGHT, Bonn, Germany, Figs. 1a-d, f,h; Figs. 1b and 1c in this article from Häfeli, H.P. 1971. Revue Suisse de Zoologie 78: 235-293, Plate VI, Fig. 2, and Plate VII, Fig. 1. G. Dentition of the lower jaw in a stage III larvae. SEM-micrograph.
may be triggered by secretions of the embryo (e.g., by prostaglandins). The epithelium in this zone continuously regenerates during the period, in which the offspring feeds. After birth of the fully transformed young, it is restored completely and looks like the normal uterine epithelium. Alterations of the uterus wall during the reproductive cycle including the zona trophica are illustrated in Fig. 12.7 and the reproductive cycle of Salamandra atra at different altitudes in Fig. 12.8 (Guex and Chen 1986; Guex and Greven 1994; Greven 1998). Guex (1994) showed that the trophic zone also is present in S. atra aurora.

The offspring feeding in the uterus stores food in its stomach (Greven and Guex 1994; Guex and Greven 1994) and defecates in the uterus (G.-D. Guex, personal communication). As yet, epitheliophagy that probably is preceded by oophagy and adelphophagy appears to be realized only in the Salamandra atra group. The phenomenon appears correlated with a long period of gestation that again is related to the duration of winter and the general low temperatures in the habitat of these species. Metamorphosis starts already at the transition from stage II to stage III and extends over a considerably long period (see Fig. 12.8).

12.5.6 Parturition

Nothing is known on factors signaling female readiness for birth. Contractions of the uterine muscularis induced by neurohypophysial hormones (12.5.1) may help to expel the young, but in Salamandra atra, a juvenile left the uterus of an anesthetized females (Häfeli 1971). A direct effect of the rich adrenergic innervation present in the uterus of S. salamandra on the control of the contraction of the uterine smooth muscle seems not probable because uterine muscles in general are spontaneously active (Greven et al. 1983).

Pieper (1970) and later Özeti (1979) recorded parturitions of Mertensiella luschani in the laboratory but did not describe the process in detail. Parturitions in Salamandra salamandra terrestris and S. salamandra salamandra take place in spring predominantly after hibernaton (e.g., Zakrzewski 1972) and have been repeatedly observed. Berweger (1926) observed a female in the shallow water with her head above the surface who rested her forelegs on the ground. After extending her hind-limbs, she gave birth to free larvae and larvae surrounded by the egg-jelly. This process was accompanied by convulsive contractions of

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Fig. 12.6 Zona trophica in pregnant Salamandra atra. A. Uterine epithelium near the zona trophica with cuboidal cells. Mitosis indicated by an arrow. B. Zona trophica at the cranial portion of the uterus. Note the irregularly shaped epithelial cells, some floating in the uterine lumen, and regions devoid of cells (arrowhead). A and B are semithin epoxy sections stained with toluidine blue-borax. C. Large epithelial cells and area free of them (arrows). TEM-micrograph. D. Intact epithelium of the zona trophica. E. Zona trophica showing protruding cells (left side) areas devoid of cells (right side). D and E are SEM-micrographs. Courtesy of Dr. G.-D. Guex. Bar for C = 10 µm, for D = 20 µm and for E = 100 µm.
the body and discharge of mucus. Birth of altogether 46 larvae and three abortive eggs lasted three days. The largest number of larvae was born on the first day. Apart from the mucus discharge and the strong convulsive contractions, these observations were often confirmed later, *i.e.*, the female resting on the fore-limbs only, and parturition of free and encased larvae covering several days. Characteristic patterns of the female immediately before and during birth are lateral spreading, rotation, and bending of the hind-limbs that elevates the body, and raising the tail. In most cases the larvae are born quickly with either the head or the tail showing first, and without or only with remains of the egg-capsule (Fig. 12.9A; *e.g.*, Szabo 1959; Greven 1976).
Fig. 12.8 Reproductive cycle of *Salamandra atra*. Mating peaks in May and June at 650 m a.s.l. and in June and July at 1700 m a.s.l. Matings can be observed, however, until the end of the season. Fertilization does not need to coincide with mating because sperm are stored in the spermathecae. Transition from stage II to stage III coincides with the formation of the zona trophica and roughly with the decline of *corpora lutea* and the resumption of oviductal and ovarian activity. From Guex, G.-D., and Greven, H. 1994. Pp. 161-208. In H. Greven and B. Thiesmeier (eds), Mertensiella Supplement 4, DGHT, Bonn, Germany, Fig. 9.
The more advanced newborn of *Salamandra salamandra bernardezi* moult within the uterus and/or during or shortly after birth. Occasionally, newborns of the Oviedo population born in captivity had long fine gills that are resorbed a short time after birth (Fig. 12.9B; Thiesmeier *et al.* 1994). Animals can stop birth in early stages of the parturition process (*S. salamandra*). In the final stage, however, a female of *S. atra* could be handled and brought into a position to take pictures (Fig. 12.9 C).

The birth process in general may be a period of danger. High mortality of female *Salamandra salamandra* during this period was already reported by Paratre (1894). Considering the poor support animals have when resting only on the fore-limbs, they may be carried away by the water current during parturition (Greven 1976).

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### 12.7 LITERATURE CITED


