REVIEW ARTICLE / PREGLEDNI ČLANAK

The influence of environmental factors on the hormonal status of lizards – a review

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Abstract

Important exogenous factors that mutually affect and regulate the annual sexual cycle of lizards, in the ecological context of adaptation, are environmental (rain, temperature, humidity, photoperiod, etc.), structural (vegetation change, food availability, etc.) and social factors (behavioural interaction, experience, etc.), while in some species endogenous (circa-annual) rhythmicity may prevail. Both endogenous and exogenous factors determine the time of hatching in the season providing the highest chances of survival for offspring. Hormonally controlled processes via the hypothalamus-pituitary-gonadal axis produce a cascade of events that determine the reproductive processes involved in reproduction, and can be found in all lizard species. These hormones, in addition to being necessary for successful reproduction and survival of the species, have numerous roles in physiology, metabolism and behaviour. The beginning, duration and end of the breeding season, and the duration of sexual inactivity, differ significantly by species and geographical distribution. Although there is much knowledge about reproduction and its regulation, there are still hidden secrets and specificities in many species that have not yet been discovered or fully explained.

Key words: *environmental factors; lizard; reproduction; sexual hormones*

Introduction

Most reptiles have reproductive particularities such as internal fertilisation, an amniotic shelled egg, complex and species-specific social behaviour, and other physiological mechanisms such as sperm storage (in some species viviparity, parental care, and in rare parthenogenesis). These traits are part of the evolutionarily developed specialisation for the purpose of survival of the species (Blackburn, 2018). Male lizards have paired copulatory organs, two penises (hemipenes) that transfer sperm from the cloaca to the female's cloaca. Females have paired ovaries and oviducts (Zwart et al., 2008). The fertilised eggs leave the female's body through the cloaca opening after a certain time (Lovern, 2011), while some lizard species are viviparous or ovoviviparous. In lizards, as in many

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other species, the integration of factors, such as environmental (rainfall precipitation, air temperature, relative humidity, photoperiod, etc.), structural (vegetation cover, vegetation change, food availability and quality, etc.) and social (behavioural interactions, experience, etc,) factors form important stimuli in controlling annual sexual cycles, observed in the ecological context of adaptation that regulates reproduction, and determines the time of hatching in the season that gives the highest changes of offspring survival (Licht, 1974; Crews, 1979; Wyk, 1994; Cruz-Cano et al., 2021; Padilla Perez and Angilletta Jr., 2022; Thonis and Akçakaya, 2024). New facts and differences in the reproduction of individual species of reptiles are discovered every day.

Seasonal breeding

Reptiles generally have precise seasonal reproductive patterns, while only a few tropical species are characterised by continuous reproduction throughout the year (Al-Amri et al., 2012). During the breeding season, due to the maturation of follicles and the preovulatory phase, females are ready to accept a male for mating (sexual receptivity) (Crews 1979), similar to seasonally polyestrous animals, e.g. small ruminants (Kovačić et al., 2023; Šavorić et al., 2024; Taherti et al., 2024). After the female has mated during that follicular cycle, often multiple times, further receptivity is inhibited in most species and subsequent males are rejected. At the end of the breeding season, most females are no longer susceptible to environmental or hormonal stimuli that can promote ovarian growth, and this refractoriness is a consequence of the atretic follicle. After the breeding season, both males and females enter a refractory or resting phase lasting several months (usually in winter), during which they are insensitive to the various environmental and social stimuli responsible for inducing gonadal recrudescence in the spring (Crews, 1979). It is assumed that in the natural environment, there is a breeding season and egg-laying during the warmer months of the year, followed by a change in temperature and daylight during the dry season in which females and males rest (Licht, 1974; Al-Amri et al., 2012). Temperature regulation in reptiles, as in all ectotherms, depends mainly on external sources (environmental temperature), which affects the development, physiology and behaviour of individuals and determines the development and survival ability of embryos during incubation (Birchard and Marcellini, 1996; Noble et al., 2018; Sanger et al., 2021; Abayarathna and Webb, 2020; De-Lima et al., 2022).

Neuroendocrine regulation of reproduction

Hormone-controlled processes are involved in reproduction process in all lizard species. These hormones play numerous roles in physiology and behaviour necessary for successful reproduction and survival of the species (Lovern, 2011). The hypothalamus-pituitary-gonadal axis produces cascade of hormones that determine reproductive processes (Sinervo and Miles, 2011; Norris and Lopez, 2018; Cermakova et al., 2023). The primary hormone from the hypothalamus is the gonadotropin-releasing hormone (GnRH), which controls gonadal activity by regulating the production and release of pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Licht, 1974; Edwards and Jones, 2017; Verderame et al., 2017). Lovern (2011) assumes that in reptiles, in addition to hormonal regulatory pathways and their connection with physiological or behavioural events, there is the phenomenon of dissociated reproductive strategies and behaviours independent of hormones.

Gonadotropins

Follicle-stimulating hormone stimulates the growth, development, and function of follicles, while LH causes follicle rupture (ovulation) and development of the *corpus luteum* (Licht, 1974). Gonadotropins (GTH) are also the main regulators of steroidogenesis and spermatogenesis. Spermatogenesis is the origin and development of the sperm cells within the male reproductive organs, the testes (Bourne and Seamark, 1975; Courty and Dufaure, 1980; Panno et al., 1992; Swain and Jones, 1994). In the testes, LH controls testosterone production by Leydig cells, while FSH controls Sertoli cell functions, including oestrogen production and active factors governing germ cell development (Verderame et al., 2017). This is a precisely regulated process that maintains a perfect balance between gonadotropins and the male (androgens) or female (oestrogens) sex steroids. External stimuli (audiovisual, olfactory, tactile) at the level of the central nervous system (CNS) can stimulate or inhibit the release of GTH, and depending on the stimulus, these will act on and modify the levels of the gonadal hormones (Licht, 1974).

Sex steroids

There are variations in plasma sex steroid levels at different stages of the reproductive cycle in lizards related to environmental factors (temperature, photoperiod, precipitation and relative humidity), and are positively associated with follicle development, while higher temperature and precipitation can be negatively associated. High concentrations of oestrogens and progesterone (P_4) were observed in late vitellogenic and preovulatory stages, and follicle development was correlated with temperature and photoperiod (Al-Amri et al., 2012). Oestrogen levels begin to rise before the start of the breeding season (at the beginning of vitellogenesis), and increases as follicles grow, and its concentration was highest in the preovulatory phase (Ando et al., 1992). After that, oestrogens remained low during the inactive phase. As steroid levels decreased, granulosa cells underwent general degeneration (Al-Amri et al., 2012).

Progesterone levels increase, which coincides with luteinization, and begin to decrease due to luteolysis and remain low throughout the inactive phase (Barbosa-Moyano et al., 2020). Low concentrations of P_4 have been reported during the early stages of follicular development, increasing follicular responses to circulating oestrogens (Duarte-Méndez et al., 2018), with higher concentrations before the onset of ovulation. Progesterone receptors were strongly expressed only during the reproductive period (Al-Amri et al., 2012; Duarte-Méndez et al., 2018). The main tissue responsible for progesterone (P_4) production in most oviparous reptiles is the *corpus luteum* (CL), while in some viviparous species the placenta is the main source of P_4 (Xavier, 1982; Blackburn, 2015). There is a direct relationship between CL development and plasma P_4 levels or between its CL degeneration and a sudden drop in plasma P_4 levels. Progesterone levels were highest during gestation to ensure successful gravidity by limiting the development of new follicles (Bonnet et al., 2001, Guerriero and Ciarcia, 2001), reducing gonadotropin synthesis (Jones, 2011), preventing contractions by modifying proteins involved in cell adhesion (Lovern, 2011; Blackburn, 2018), facilitating maternal recognition during gestation, and promoting the expulsion of eggs or offspring in viviparous reptiles (Brandley et al., 2012; Barbosa-Moyano et al., 2020).

Similarly, reproductive processes in males are neuro-hormonally regulated, and hormone levels have a seasonal character. The Southern Hemisphere has the opposite seasons from the Northern, so winter in the Southern Hemisphere corresponds to summer, and spring to autumn in the Northern Hemisphere. The border of changing seasons shifts towards the equator in tropical and subtropical areas. In tropical regions, oestrogen levels begin to increase before the start of the breeding season (at the beginning of vitellogenesis) and continue to rise between February and May, in the autumn, during ovulation, gestation and egg laying). During the winter, *i.e.*, the sexually inactive phase (June-August), the level of oestrogen is low. The level of progesterone increases significantly in March and April (during luteinization), then begins to decrease in May to June due to luteolysis, and remains low during the entire phase of sexual rest (Motta et al., 2020). The breeding season begins when testosterone levels rise, which determines the production of a certain amount of sperm that travels from the testicles to the epididymis to be ejaculated. Male sex hormones in plasma and testes had higher levels during spermatogenesis, and also affected the growth and mating behaviour of lizards (Bourne and Seamark, 1975; Mckinney and Marion, 1985; Panno et al., 1992; Phillips and Millar, 1998; Cox and John-Alder, 2005). Oestrogen levels increase in males of some species, ending the breeding season. During this period, the seminiferous tubules show only spermatogonia and Sertoli cells. At the beginning of the next season, spermatogenesis starts again (Verderame et al., 2017). Males living in environments with more pronounced seasonal differences exhibit testicular regression during the dry season and recrudescence before the wet season (Phillips and Millar, 1998). The beginning, duration and end of the breeding season, as well as the duration of the season of sexual inactivity, differ significantly among species and by geographical distribution (Otero et al., 2015). Geographic differences in reproduction may be due to maternal effects varying with latitude, and a significant influence on reproductive performance could be influenced by maternal diet and energy accumulation of the previous season or before reproduction during the same season (Du et al., 2014).

Conclusion

Most lizards, even in tropical regions, show obvious seasonal changes in reproductive activity, and similar to most species from temperate regions, there is a period of sexual rest after the breeding period. Exogenous, climatic factors act to synchronise these cycles, although some species may show endogenous (circa-annual) rhythmicity. Climate change can be potentially dangerous for the reproduction of many lizard species, so it is necessary to have a good understanding of how environmental factors impact growth, development and reproduction, and to strengthen efforts to develop more effective strategies to improve species conservation. Ideal temperature is the most important stimulus for reproduction, higher humidity (precipitation) can affect egg laying in tropical regions, while, in some species, the influence of photoperiodism is uncertain. The gonadotropins are most susceptible to the influence of environmental factors (excessively high environmental temperatures and photoperiod in some species), and these hormones can be modified according to stimuli processed in the CNS. Gametogenesis and steroidogenesis can be affected differently by changes in temperature, and sensitivity is species-dependent. Knowledge about the reproduction of lizards and the neuroendocrine regulation of the sexual cycle, especially in some species, is scarce, and many facts still need to be discovered and confirmed.

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Utjecaj okolišnih čimbenika na hormonalni status guštera – pregledni rad

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Važni egzogeni čimbenici koji međusobno utječu i reguliraju godišnji spolni ciklus guštera, u ekološkom kontekstu prilagodbe, su: okolišni (kiša, temperatura, vlaga, fotoperiod i dr.), strukturni (promjena vegetacije, dostupnost hrane i dr.) i društveni čimbenici (interakcija ponašanja, iskustvo itd.), a kod nekih vrsta može prevladati i endogena (cirkaanualna) ritmičnost. Endogeni i egzogeni čimbenici određuju vrijeme izlijeganja u sezoni u kojoj je preživljavanje potomaka najveće. Hormonski kontrolirani procesi preko osovine hipotalamus-hipofiza-gonade proizvode kaskadu događaja koji određuju reproduktivne procese uključene u reprodukciju, a mogu se naći kod svih vrsta guštera. Ovi hormoni, osim što su nužni za uspješnu reprodukciju i opstanak vrste, imaju brojne uloge u fiziologiji, metabolizmu i ponašanju. Početak, trajanje i kraj sezone parenja, kao i trajanje sezone spolne neaktivnosti, značajno se razlikuju ovisno o vrsti i geografskoj rasprostranjenosti guštera. Iako postoje mnoga saznanja o razmnožavanju gmazova i njegovoj regulaciji, još više skrivenih tajni i specifičnosti kod mnogih vrsta, nisu otkrivene niti do kraja razjašnjene.

Ključne riječi: *čimbenici okoliša, gušteri, razmnožavanje, spolni hormoni*