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Prostaglandins and Sex Steroids from Reptilian (*Podarcis sicula sicula*) Ovarian Follicles at Different Developmental Stages

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ABSTRACT—Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin E_2 (PGE_2), progesterone, androgens, and estradiol-17β *in vitro* basal release by follicles of the oviparous lizard, *Podarcis s. sicula* was studied; in addition, the *in vitro* effect of $PGF_{2\alpha}$ and PGE_2 on sex steroid release was evaluated. Follicles were divided according to the different vitellogenic developmental stages: pre-vitellogenic, early-vitellogenic, mid-vitellogenic and fully-grown. $PGF_{2\alpha}$ and progesterone basal release was highest in fully-grown follicles; PGE_2 and estradiol basal release was highest in early-vitellogenic follicles; androgens basal release was detectable in mid-vitellogenic and fully-grown vitellogenic follicles only. $PGF_{2\alpha}$ increased progesterone release by fully-grown follicles; PGE_2 increased estradiol release by all follicle types, except by early-vitellogenic ones. The present data suggest that $PGF_{2\alpha}$ and PGE_2 exert different roles on follicles: $PGF_{2\alpha}$ seems to induce ovulation through the mediation of progesterone, while PGE_2 seems to be implied in the start and the sustaining of oocyte vitellogenic development through the mediation of estradiol.

INTRODUCTION

In mammals, prostaglandins (PGs) of both F and E series modulate the ovary function, including steroidogenesis [6, 9, 18, 29]. PGs intervene in ovulation and oviduct relax in birds [1, 30]. As regards oviparous reptiles, oviposition is correlated with a dramatic elevation of prostaglandin F (PGF) and prostaglandin E_2 (PGE_2) in the loggerhead turtle, *Caretta caretta* [20] and the tuatara, *Sphenodon punctatus* [19]; $PGF_{2\alpha}$ exhibits luteolytic effects in the lizard, *Anolis carolinensis* [21], and the snapping turtle, *Chelydra serpentina* [24], determining a decline in plasma progesterone. Little is known about the involvement of PGs in the vitellogenic development of follicles in reptiles, and, in particular, nothing is known about the role of PGs on the reproductive processes of the oviparous lizard, *Podarcis sicula sicula,* even if the reproductive cycle of this lizard has been well studied [2, 3, 10].

In this study we have compared the $PGF_{2\alpha}$, PGE_2, progesterone, androgens, and estradiol-17β *in vitro* basal releases by follicles, at different vitellogenic developmental stages, of the lizard, *Podarcis s. sicula*; in addition, the *in vitro* effect of $PGF_{2\alpha}$ and PGE_2 on progesterone, androgens, and estradiol-17β releases by the same follicles has been examined.

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MATERIALS AND METHODS

Animals The reproductive cycle of the female lizard, *Podarcis s. sicula,* living in the sourroundings of Naples (Campania, Italy, 25–75 m above sea level) is here briefly described. From the end of summer to the beginning of the next spring (stasis), gonads and genital apparatus are quiescent; then, soon after emerging, the ovary and oviduct undergo recovery and reach their full growth from March to mid-May (recovery period);

ovulation and egg deposition occur from mid-May to July (ovulatory period); after deposition, gonads and oviduct regress (postovulatory period) [2, 3, 10].

Adult female lizards, *Podarcis s. sicula,* of this population were captured in May and transferred to laboratory terraria, maintained in ambient photothermal conditions (L/D: 15/9; 18°C), and fed with meal worms and fresh vegetables *ad libitum.*

In vitro incubations of follicles The lizards were sacrificed by decapitation, the developmental stage of the reproductive organs was observed and the ovaries rapidly removed. Follicles were freed under dissection microscope and placed in cold Dulbecco's modified Eagle medium (DME, Sigma, USA) containing 10 mM Hepes, 1 mg Penicillin/ml and 2 mg Streptomycin/ml. Follicles were divided according to the different vitellogenic developmental stages: pre-vitellogenic (500–1000 μm ϕ), early-vitellogenic (1500–3000 μm ϕ), mid-vitellogenic (4000–6000 μm ϕ) and fully-grown (>8000 μm ϕ). The follicles were placed in multiple tissue culture plates (Beckton Dickinson, USA); each well contained one follicle. Six follicles of each type were divided into 3 experimental groups (each consisting of 2 wells). The experimental groups were: a) follicle with DME alone; b) follicle with DME plus $PGF_{2\alpha}$ (150 ng); c) follicle with DME plus PGE_2 (150 ng). The final volume of each well was 1 ml. Culture plates were wrapped with aluminium foil and incubated in a shaking water bath (32°C), set at 30 rpm. One well of each experimental group was removed, respectively, after 3 and 6 hr of incubation. The incubation medium samples were immediately stored at −20°C for later hormone determination. In addition, the experiment was repeated without follicles. Tests on 5 parallel incubation sets were carried out. Preliminary evidence led our choosing to the incubation conditions and the $PGF_{2\alpha}$ or PGE_2 minimum effective dose utilized in the present *in vitro* study (data not shown).

$PGF_{2\alpha}$, PGE_2, progesterone, androgens, and estradiol-17β determination $PGF_{2\alpha}$ and PGE_2 were determined in incubation medium samples by radioimmunoassay (RIA) [12, 13]. The determinations were carried out on duplicate incubation medium samples (500 μl) that were extracted with 5 ml of diethyl ether for 4 min. The organic fractions were transferred into glass tubes and evaporated to dryness under a nitrogen stream. The extracts were resuspended with 1 ml of assay buffer and assayed. The recovery of labelled $PGF_{2\alpha}$ and PGE_2 were 83.3±1.3% and 88.8±1.7%, respectively. Parallelism among the standard curve in buffer, a standard curve in incubation medium (then extracted) and a serial dilution of a single medium sample (extracted) were constant.

Concentrations of progesterone, androgens and estradiol-17β in incubation media were determined by RIA in accordance with methods previously reported [7, 13].

The following sensitivities were recorded: $PGF_{2\alpha}$ 13 pg (intraassay variability: 7.5%; interassay variability: 14.5%); PGE_2, 15.5 pg (intraassay variability: 7.0%; interassay variability: 12.5%); progesterone, 9 pg (intraassay variability: 8.0%; interassay variability: 10.0%); androgens, 7.5 pg (intraassay variability: 6.0%; interassay variability: 11.5%); estradiol-17β, 7 pg (intraassay variability: 8.5%; interassay variability: 13.0%). $PGF_{2\alpha}$, progesterone, testosterone, and estradiol-17β antisera were provided by Dr. G. F. Bolelli and Dr. F. Franceschetti (CNR-Physiopathology of Reproduction Service, University of Bologna, Italy). The PGE_2 antiserum was purchased from Cayman Chemical (USA). Testosterone was not separated from 5α-dihydrotestosterone; therefore, the antiserum used is not specific and the data are expressed as androgens. Tritiated $PGF_{2\alpha}$, PGE_2, progesterone, testosterone, and estradiol-17β were purchased from Amersham International (UK) and non-radioactive $PGF_{2\alpha}$, PGE_2, progesterone, testosterone, and estradiol-17β from Sigma.

Statistics Data relative to each hormone were submitted to analysis of variance (ANOVA) followed by Duncan's multiple range test [8, 31]. Correlation coefficients followed the procedures of Scossiroli and Palenzona [28].

RESULTS

$PGF_{2\alpha}$ basal release was higher ($P<0.01$) in fully-grown follicles with respect to the other follicle types; $PGF_{2\alpha}$ was lower ($P<0.01$) in pre-vitellogenic with respect to early-vitellogenic and mid-vitellogenic follicles (Fig. 1). PGE_2 basal release was higher ($P<0.01$) in early-vitellogenic with respect to the other follile types; PGE_2 was lower in pre-vitellogenic with respect to mid-vitellogenic and fully-grown follicles (Fig. 2). Progesterone basal release was higher ($P<0.01$) in fully-grown with respect to the other follicle types; progesterone was higher ($P<0.01$) in mid-vitellogenic with respect to the pre-vitellogenic and early-vitellogenic follicles; progesterone was higher ($P<0.01$) in early-vitellogenic with respect to pre-vitellogenic follicles (Fig. 3, upper panel).

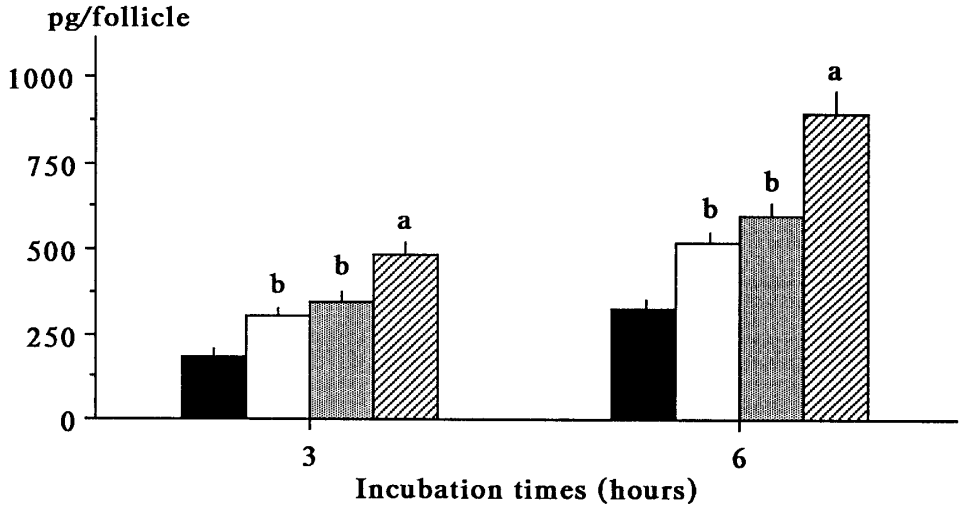

FIG. 1. $PGF_{2\alpha}$ basal release by follicles, at different vitellogenic developmental stages (see Materials and Methods), of the oviparous lizard, *Podarcis s. sicula*. Follicles: pre-vitellogenic (black bars), early-vitellogenic (white bars), mid-vitellogenic (grey bars), fully-grown (hatched bars). Each mean refers to 5 values ± SD. a, $P<0.01$ vs pre-vitellogenic, early-vitellogenic and mid-vitellogenic follicles; b, $P<0.01$ vs pre-vitellogenic follicles (Duncan's multiple range test).

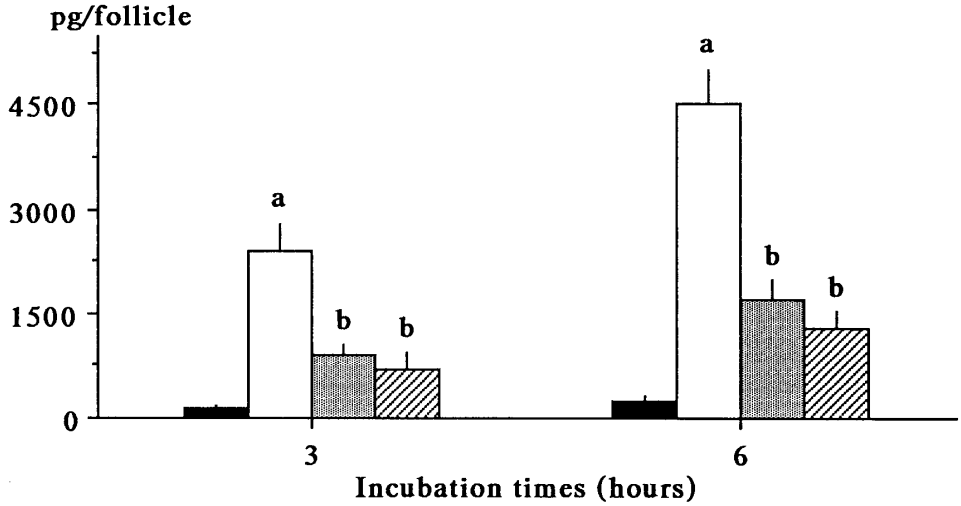

FIG. 2. PGE_2 basal release by follicles, at different vitellogenic developmental stages (see Materials and Methods), of the oviparous lizard, *Podarcis s. sicula*. Follicles: pre-vitellogenic (black bars), early-vitellogenic (white bars), mid-vitellogenic (grey bars), fully-grown (hatched bars). Each mean refers to 5 values ± SD. a, $P<0.01$ vs pre-vitellogenic, mid-vitellogenic and fully-grown follicles; b, $P<0.01$ vs pre-vitellogenic follicles (Duncan's multiple range test).

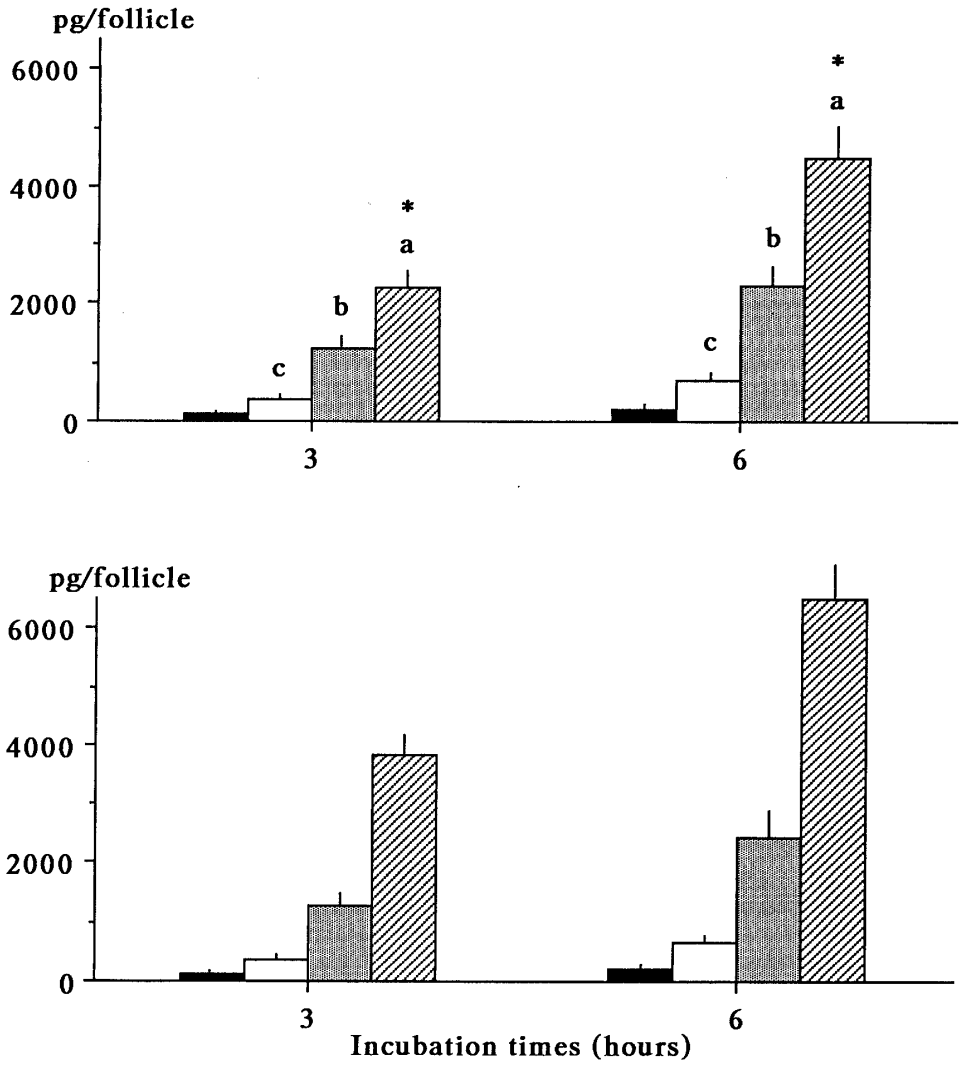

FIG. 3. Progesterone basal release (upper panel) and $PGF_{2\alpha}$ effects (lower panel) on progesterone release by follicles, at different vitellogenic developmental stages (see Materials and Methods), of the oviparous lizard, *Podarcis s. sicula*. Follicles: pre-vitellogenic (black bars), early-vitellogenic (white bars), mid-vitellogenic (grey bars), fully-grown (hatched bars). Each mean refers to 5 values $\pm$ SD. a, $P<0.01$ vs pre-vitellogenic, early-vitellogenic and mid-vitellogenic follicles; b, $P<0.01$ vs pre-vitellogenic and early-vitellogenic follices; c, $P<0.01$ vs pre-vitellogenic follicles; $^*P<0.01$ vs the same follicle type incubated with $PGF_{2\alpha}$ (Duncan's multiple range test).

Androgens basal release was not detectable in pre-vitellogenic and early-vitellogenic follicles; androgens were higher ($P<0.01$) in fully-grown with respect to mid-vitellogenic follicles (Fig. 4). Estradiol-17β basal release was higher ($P<0.01$) in early-vitellogenic with respect to the other follicle types; estradiol was lower ($P<0.01$) in pre-vitellogenic with respect to mid-vitellogenic and fully-grown follicles (Fig. 5, upper panel). $PGF_{2\alpha}$ basal release values were positively correlated

FIG. 5. Estradiol-17β basal release (upper panel) and PGE_2 effects (lower panel) on estradiol-17β release by follicles, at different vitellogenic developmental stages (see Materials and Methods), of the oviparous lizard, *Podarcis s. sicula*. Follicles: pre-vitellogenic (black bars), early-vitellogenic (white bars), mid-vitellogenic (grey bars), fully-grown (hatched bars). Each mean refers to 5 values $\pm$ SD. a, $P<0.01$ vs pre-vitellogenic, mid-vitellogenic and fully-grown follicles; b, $P<0.01$ vs pre-vitellogenic follicles; $^*P<0.01$ vs the same follicle type incubated with PGE_2 (Duncan's multiple range test).

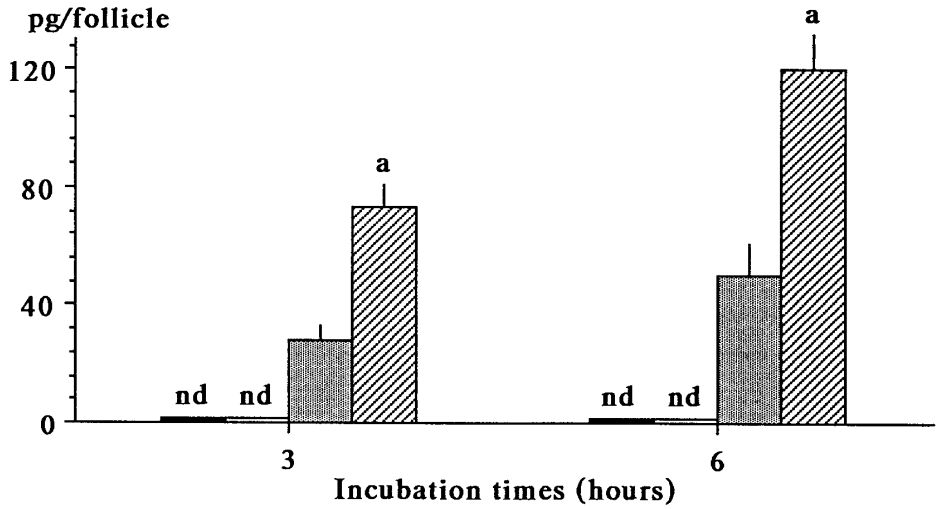

FIG. 4. Androgens basal release by follicles, at different vitellogenic developmental stages (see Materials and Methods), of the oviparous lizard, *Podarcis s. sicula*. Follicles: pre-vitellogenic (black bars), early-vitellogenic (white bars), mid-vitellogenic (grey bars), fully-grown (hatched bars). Each mean refers to 5 values ± SD. a, $P <$ 0.01 vs mid-vitellogenic follicles (Duncan's multiple range test); nd, not detectable.

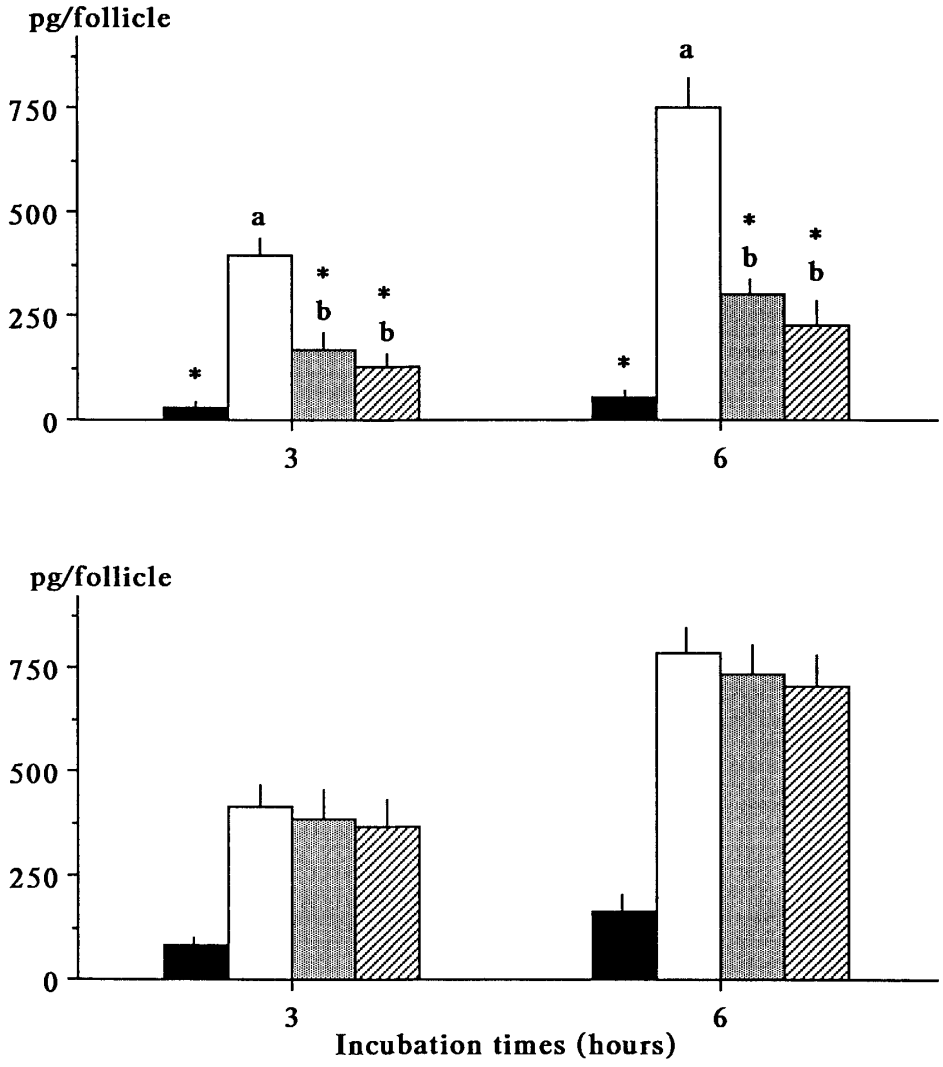

TABLE 1. Correlation coefficents among $PGF_{2\alpha}$, PGE_2, progesterone and estradiol-17β levels released by follicles, at different vitellogenic developmental stages, of *Podarcis s. sicula*

	Incubation times	
	3 hr	6 hr
$PGF_{2\alpha}$ vs progesterone	0.804	0.814
PGE_2 vs estradiol-17β	0.862	0.911

All correlations show the same level of significance ($P<0.001$); df=18.

($P<0.001$) to those of progesterone (Table 1); PGE_2 basal release values were positively correlated ($P<0.001$) to those of estradiol-17β (Table 1).

$PGF_{2\alpha}$ induced an increase ($P<0.01$) in progesterone release by fully-grown follicles (Fig. 3, lower panel). PGE_2 induced an increase ($P<0.01$) in estradiol release by pre-vitellogenic, mid-vitellogenic, and fully-grown follicles (Fig. 5, lower panel).

DISCUSSION

This is the first study regarding the *in vitro* basal release of PGs and sex steroids by the follicles, kept at different vitellogenic developmental stages, of the oviparous lizard *Podarcis s. sicula.*

$PGF_{2\alpha}$ showed the highest amounts in fully-grown follicle incubation samples; this finding supports the idea that $PGF_{2\alpha}$ could be involved in the stimulation of ovulation; on the other hand, Goetz [15] reported that in several fish species, PGs can induce ovulation; in particular, plasma and ovarian tissue $PGF_{2\alpha}$ levels are higher during ovulation in the yellow perk, *Perca flavescens* [17], in the brook trout, *Salvelinus fontinalis* [16], in the goldfish, *Carassius auratus* [4], and in the pond loach, *Misgurnus anguillicaudatus* [25]. Also in amphibians, $PGF_{2\alpha}$ seems to be implied in ovulation processes, seeing that $PGF_{2\alpha}$ *in vitro* induces ovulation in *Rana pipiens* [27]; more recently Gobbetti and Zerani [14] suggested that $PGF_{2\alpha}$ is implied in the control of ovulation in *Rana esculenta.* The involvement of $PGF_{2\alpha}$ in the ovulatory process of this lizard is also supported by the stimulatory role exerted by this prostaglandin on progesterone increase by fully-grown follicles. Progesterone basal release was higher in fully-grown follicles, if compared with the other follicle types, so suggesting that this steroid is involved in the ovulation of *Podarcis,* as many authors reported for amphibian species [23]; in fact, progesterone, produced by follicle cells, induces ovulation in *Xenopus laevis* [32] and *Rana pipiens* [26].

The early-vitellogenic follicles released the highest values of PGE_2 and estradiol, which both, however, remained still high in mid-vitellogenic and fully-grown follicles. The positive correlation between the basal release values of PGE_2 and estradiol suggests a causal relationship and this hypothesis is validated by the PGE_2 effect in increasing estradiol release. This finding might indicate a possible trigger role for this prostaglandin in the start of the vitellogenic development of the oocytes and also a role in sustaining the following vitellogenic growth of oocytes. In this context we recall that estradiol induces the hepatic vitellogenin synthesis for the development of the follicle, as widely reported for reptiles [5, 22]. The PGE_2 stimulatory effect on estradiol release was detected in all follicle types, except for early-vitellogenic ones. This phenomenon could be due to the refractoriness of this follicle type to an additional stimulation of PGE_2; in fact the early-vitellogenetic follicles released the highest amounts of PGE_2 and estradiol.

Androgens were detected only in the incubation media of mid-vitellogenic and fully-grown follicles in accordance with Fortune [11], who found that only large follicles released these hormones *in vitro* in the amphibian *Xenopus laevis.* The meaning of this last result is unclear; it could be due to the catabolism of progesterone which exhibits the highest values in incubation media of mid-vitellogenic and fully-grown follicles; however, a role for androgens produced by these two follicle types cannot be excluded.

Summarizing, this work suggests different roles for $PGF_{2\alpha}$ and PGE_2 released by *Podarcis s. sicula* follicles: $PGF_{2\alpha}$ could induce ovulation through progesterone mediation, while PGE_2 could be implied in the start and the sustaining of vitellogenic development of oocytes through estradiol mediation.

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