Received: 4 June 2018 | Accepted: 27 July 2018

DOI: 10.1002/jez.2222

RESEARCH PAPER

WILEY JEZ-A ECOLOGICAL AND INTEGRATIVE PHYSIOLOGY

Dynamics of steroid-induced oocyte maturation in three amphibian species: Mathematical modeling and simulation

Carolina Manzano[1] | Maria Graciela Benzal[2] | Liliana Isabel Zelarayán[3]

[1]PROIMI – Biotecnología, CONICET, Tucumán, Argentina

[2]Facultad de Bioquímica, Química y Farmacia, Instituto de Matemática, Tucumán, Argentina

[3]Facultad de Bioquímica, Química y Farmacia, INSIBIO CONICET-UNT, Tucumán, Argentina

Correspondence
Carolina Manzano, PROIMI – Biotecnología, CONICET. Av. Belgrano y Pje, Caseros, 4000 Tucumán, Argentina.
Email: caro.manzano91@gmail.com

Abstract

Oocyte maturation in vertebrates involves morphological, biochemical, and functional changes through which meiosis, previously arrested in Profase I, is resumed. Within these changes, germinal vesicle breakdown (GVBD) constitutes morphological evidence of the resumption of the cellular cycle. Sex steroids (progestins and androgens) play a key role in oocyte maturation and are considered inducers of this process. The aims of this study were to analyze the effect of sex steroids on the oocyte maturation of amphibians and to model and simulate the dynamics of this process through the experimental data obtained. The oocytes of sexually mature females of *Scinax fuscovarius*, *Pleurodema borellii*, and *Physalaemus biligonigerus* were treated with different concentrations (0.001–1 μM) of sex steroids progesterone (P_4) and testosterone (T) for 24 hr. Dose– and time–response curves were performed with the results achieved. Sex steroids induced oocyte maturation in a dose-dependent manner in all three species. The dose at which the highest maturation percentage was found was 1 μM. No significant differences were observed between GVBD percentages of P_4 and T. However, time–response curves show that oocytes responded earlier and achieved higher GVBD percentages when treated with P_4. Gompertz mathematical model was proposed in this study to simulate GVBD dynamics. This model properly fits the corresponding experimental data and allows the analysis of the behavior of oocyte maturation in amphibians and the quantification of parameters with biological meaning that are indicative of sex steroids efficiency in this process.

KEYWORDS

amphibians, Gompertz model, oocyte maturation, sex steroids

1 | INTRODUCTION

In amphibians, reproduction is a process whose synchronization requires coordination between environmental, physiological, and behavioral signals (Vu & Trudeau, 2016). The secretion of hypophyseal gonadotropins (Gns) stimulates two ovarian functions, oogenesis, and steroidogenesis, in a seasonal manner (Arias Torres, Bühler, & Zelarayán, 2016; Norris & López, 2011). During the reproductive period, moments before ovulation, the increase in Gns induces follicular cells to produce the maturation-inducing steroid (MIS). Oocytes maturation or meiotic resumption is evidenced by the nucleus or germinal vesicle breakdown (GVBD). Oocytes arrested in Prophase I complete the first meiotic division and are arrested again in metaphase II (Bühler, Sánchez Toranzo, & Zelarayán, 2014; Marteil, Richard-Parpaillon, & Kubiak, 2009). Thus, under tight gonadotropic control, the ovary regulates the development and release of mature oocytes ready for successful fertilization.

Sex steroid production in amphibians is essential for follicle growth and oocyte maturation. Numerous published works have suggested that progesterone (P_4) synthesized by ovarian follicles in response to Gns interacts with the oocyte surface to trigger a complex chain of

morphological and biochemistry events that induce GVBD in vitro (Maller, 2001; Zelarayán, Ajmat, Bonilla, & Bühler, 2013). For several decades, P_4 was the MIS in amphibian oocytes (Smith, 1989). More recently, however, other steroid hormones have been considered as physiological inducers of this process. Among them, testosterone (T) is considered as one of the main steroids produced by the ovarian follicles of *Xenopus laevis* responsible for GVBD in this species (Deng et al., 2009; Sen, Prizant, & Hammes, 2011).

In *Rhinella arenarum* oocytes, P_4 has been identified as the main steroid responsible for maturation (Arias Torres, Buhler, & Zelarayán, 2016; Arias Torres et al., 2017). However, during the reproductive period, plasma levels of P_4 in *R. arenarum* are low at the time of ovulation (Medina, Ramos, Crespo, González-Calvar, & Fernández, 2004), while androgens have elevated plasma levels during this stage. In agreement with the above, some authors consider that androgens play a central role in the maturation of *X. laevis* oocyte (Deng et al., 2009; Rasar & Hammes, 2006).

Although *X. laevis* oocytes have been considered a good biological model to study the events that take place during their maturation, the reproductive strategies of amphibians are multiple. Consequently, the study of the reproductive biology of native species of South America not yet studied such as *Scinax fuscovarius*, *Pleurodema borellii*, and *Physalaemus biligonigerus* is of interest.

Traditionally, the oocyte maturation process has been studied with an experimental approach. However, experimental research can be complemented with mathematical modeling and simulation. Mathematical models of population dynamics are widely used to describe the behavior of biological processes. These models can be used in various areas of interest such as tumor and bacterial growth (Cayre, Vignolo, & Garro, 2001; Menchón, 2007) and animal production (Alvarez, Quintana, Mallo, & Quinn, 2005; Casas, Rodríguez, & Téllez, 2010), among others.

The description of a real phenomenon or process in mathematical terms is known as mathematical modeling. It consists of proposing a mathematical model that relates the variables and parameters of interest and that allows the description of its fundamental aspects in a simple and precise way. In any case, the result obtained from the proposed mathematical model must be justified by experimental data to validate it (Benzal, 2003; Glynn, Unudurthi, & Hund, 2014).

There is a great variety of mathematical models, and their choice depends on the phenomenon under study. For example, classical models of population dynamics such as Gompertz (Karkach, 2006; Nobile, Ricciardi, & Sacerdote, 1982) analyze the growth in a number of individuals within a population through the parameters that determine them. Particularly, the amphibian ovary can be thought of as a population where the set of follicles that compose it can be analyzed as a group of individuals going through different phases of the physiological process of maturation. During these stages, the germinal vesicle undergoes conformational changes that involve the breakdown of the nuclear envelope (represented by the percentage of GVBD) and the rearrangement of chromosomes until metaphase II is achieved (Benzal & Zelarayán, 2015).

Although numerous fields in biology apply mathematical models, so far there are few works on reproductive physiology that use them as a tool, so it is of interest in this paper to present the study of GVBD dynamics from an initial (hormone induction) to a final time (oocytes in metaphase II or mature), using GVBD percentages as a reference. This study allows quantification of parameters with biological meaning associated with different phases of maturation, between initial and final time. The dynamics of the changes that occur during maturation are analyzed in accordance with the mathematical properties of the Gompertz model of sigmoidal growth.

To strengthen the link between mathematics and biology, this study shows results from experimentation, modeling, and simulation of the dynamics of maturation induced by sex steroids P_4 and T in ovarian follicles of *S. fuscovarius*, *P. borellii*, and *P. biligonigerus*.

2 | MATERIALS AND METHODS

2.1 | Experimental methods

Sexually mature females of *S. fuscovarius*, *P. borellii*, and *P. biligonigerus* were collected in Tucumán, northwestern Argentina, during the reproductive period (October to March). Collected animals were kept in captivity for a few hours. Three females of each species were subsequently anesthetized and operated for the extraction of ovaries in accordance with the Guiding Principles for the Care and Use of Research Animals of the Society for the Study of Reproduction.

Amphibian Ringer (AR) solution (6.60 g NaCl/L, 0.15 g $CaCl_2$/L, and 0.15 g KCl/L, pH 7.4) with penicillin G sodium (30 mg/L) and streptomycin sulfate (50 mg/L) was used as a culture medium in all routine incubations.

All procedures were performed under a stereoscopic microscope at room temperature (24°C). Tissue manipulation was done manually using watchmaker's tweezers (Zelarayán, Oterino, & Bühler, 1995). Fully grown follicles were selected and incubated in duplicate or triplicate at a controlled temperature of 26°C. Routine incubations were carried out in vitro in disposable multiwell plastic boxes. A total of 15–20 follicles were incubated in 2 ml of AR. Steroids P_4 and T were incorporated directly into the incubation medium at a 5-μl volume.

The criterion for maturation was GVBD, judged by the appearance of a white spot surrounded by a pigmented area around the animal pole during the first few hours after hormone treatment and by GVBD as detected by dissecting the samples under a stereomicroscope, after fixation in Ancel and Vintemberger's solution (10% formol, 0.5% acetic acid, and 0.5% NaCl) overnight at room temperature. Maturation rate was expressed as a percentage of GVBD.

Dose–response curves of steroids were performed by incubating follicles for 22–24 hr in the presence of different doses of the steroids (0.001–1 μM). Time–response curves of steroids (0–24 hr) were modeled and simulated using the mathematical software MATLAB (The MathWorks Inc., 2008).

2.2 | Statistical analysis

Experimental results of the effect of sex steroids on oocyte maturation are expressed as mean ± standard error of mean (*SEM*).

Comparisons between treatments were carried out using Student's *t*-test and Tukey's Studentized Range Test to determine if there were statistical differences at the 0.05 significance level.

2.3 | Mathematical properties of the Gompertz model

Mathematical modeling, along with simulation, allows the adjustment of a model and constitutes a procedure that feeds back to obtain the model that best explains and describes the real phenomenon (Glynn et al., 2014). In this study, the Gompertz model of population dynamics was used to analyze the dynamics of GVBD. It is a self-regulated growth model where the growth rate exponentially decreases over time. It is frequently used in biology to describe individual growth and is formulated by the ordinary nonlinear differential equation:

$$\frac{dx}{dt} = r \times \ln\left(\frac{K}{x}\right), \tag{1}$$

where the variable $t \geq 0$ represents time measured in hours and the variable $x \geq 0$ stands for the GVBD percentage at time t. The parameter r (hr^{-1}); $r > 0$ represents the intrinsic growth rate associated with the speed at which GVBD occurs. Parameter K; $0 < K \leq 100$ is associated with the highest GVBD percentage achieved in the steady state (within 24 hr).

The Gompertz model has two equilibrium points: $x = 0$ and $x = K$ so that $x = 0$ is unstable, whereas $x = K$ is asymptotically stable. As there are no other equilibrium points, any population evolves away from zero and asymptotically approaches K.

Gompertz model (Equation (1)) can be integrated exactly, so the solution of the Gompertz model is the Gompertz function, wich depends on the exponential function (exp), in the following way:

$$x(t) = K \exp\ (-\beta \exp(-rt)), \tag{2}$$

where

$$\beta = e^{-r\tau}. \tag{3}$$

Parameters r and K of the Gompertz model (Equation (1)) intervene in Equation (2) and a third parameter $\beta > 0$ (Equation (3)) controls the difference between the initial value and the end of the GVBD percentage. The three parameters r, K, and β are related at the inflection point (τ, x_τ) of the solution path given by Equation (2), so that the inflection time $\tau = \ln\beta/r$ is obtained from Equation (3) and the corresponding level of inflection $x_\tau = K/e$ is obtained from mathematical analysis of Equations (1) and (2).

On the other hand, from the Gompertz model (Equation (1)) it follows that the relative growth rate is:

$$\frac{1}{x}\frac{dx}{dt} = r\ln\left(\frac{K}{x}\right) = -r\ln(x) + r\ln(K) = -\ r\ln(x) + b, \tag{4}$$

where

$$b = r\ln(K). \tag{5}$$

Taking Equation (4) into account, experimental data was expressed in the form $(1/x)(dx/dt)$ to obtain a first estimate of parameters r and b that fits $(1/x)(dx/dt) = -r\ln(x) + b$, so a first estimate of the K parameter is obtained from Equation (5), that is:

$$K = \exp\left(\frac{b}{r}\right). \tag{6}$$

Values obtained for these parameters were used to apply the NLIN procedure of SAS (Statistical Analysis System) (SAS, 2004) that fits nonlinear regression models and estimates the parameters by nonlinear least squares or weighted nonlinear least squares.

The use of nonlinear regression analysis is indicated when the functional relationship between the response variable and the predictor variables is nonlinear, as in the case of the Gompertz model. Nonlinearity in this context refers to a nonlinear relationship between the parameters.

Simulation has allowed the adjustment of the Gompertz model because it is a procedure that feeds back to obtain a model that best explains and describes the real biological system or process (Glynn et al., 2014). In this study, a better adjustment of parameters r, K, and β of Equation (2) with MATLAB was made by implementing an algorithm that considers the Gompertz model, the experimental data and the NLIN procedure. They allow the obtainment of the level of inflection $x_\tau = K/e$ that occurs at the time of inflection given by:

$$\tau = \frac{\ln\beta}{r}. \tag{7}$$

The inflection time corresponds to the moment when the growth rate of GVBD is maximal. From this moment the growth rate begins to decrease until it is annulled in the steady state. The Gompertz curve generates asymmetry around the inflection point (τ, x_τ), which is reached before 50% of the K value (maximum GVBD percentage), more precisely for an $x_\tau \leq 36.7\%$ of GVBD.

In this study, the simulation process was conducted with MATLAB by means of an executable algorithm that was carried out including the experimental data and the values of parameters r, K, and β adjusted with NLIN. In addition, it has been imposed the condition that the solution trajectory of the Gompertz model passes through the inflection point (τ, x_τ). Thus, an adequate simulation of GVBD dynamics is obtained for 24 hr, whose behavior is analyzed qualitatively by visualizing the corresponding graphs.

3 | RESULTS

3.1 | Effect of P_4 and T on nuclear maturation

The effect of sex steroids P_4 and T was tested in fully grown follicles of *S. fuscovarius*, *P. borellii*, and *P. biligonigerus* during the reproductive period. Follicles of the three species resumed meiosis in the presence of steroids at all doses tested (0.001–1 μM).

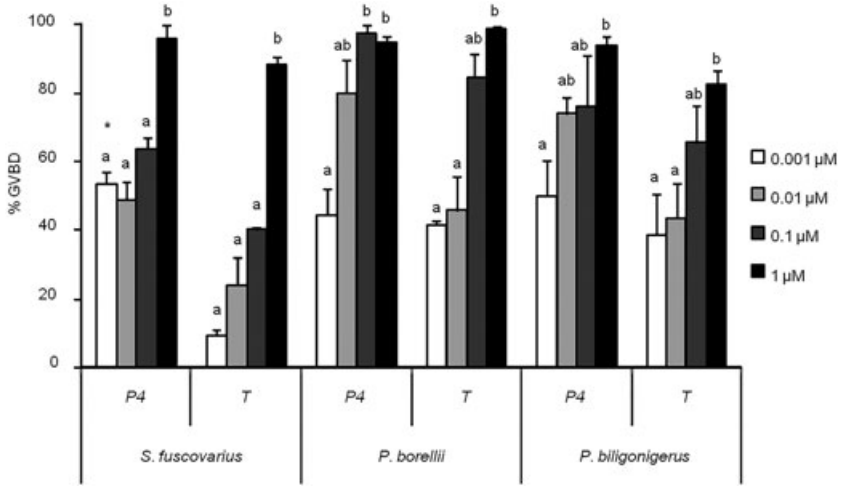

FIGURE 1 Effect of P_4 and T on follicle maturation of (a) *S. fuscovarius*, (b) *P. borellii* and (c) *P. biligonigerus*. Follicles were incubated in the presence of increasing doses of P_4 and T (0.001–1 µM). GVBD was controlled after 24 hr of incubation. Error bars indicate ±*SEM* ($n = 4$). Different letters indicate significant differences ($P < 0.05$) between doses for each category. Asterisks indicate significant differences in GVBD between P_4 and T for each dose. GVBD: germinal vesicle breakdown; P_4: progesterone; *SEM*: standard error of the mean; T: testosterone

Treatment of follicles with P_4 and T stimulated GVBD in a dose-dependent manner. With both steroids, the dose at which the highest GVBD percentage was reached was 1 µM (92.7 ± 2.5%), whereas follicles treated with a 0.001 µM dose matured in a variable way (Figure 1). Comparatively, when analyzing the effect of each hormone on follicles maturation, we observed that, when treated with P_4, 49.2 ± 2.7% of the follicles resumed meiosis with doses of 0.001 µM and 95.1 ± 0.4% of the follicles did so with doses of 1 µM in the three species. In contrast, follicles treated with T (0.001 µM) obtained 29.6 ± 10.3% of GVBD.

In *S. fuscovarius* follicles treated with 1 µM of T exceeded 50% of GVBD, whereas in *P. borellii* and *P. biligonigerus* the dose required to reach the same result was 0.1 µM (Figure 1).

Although a greater biological response was observed in follicles treated with P_4, this was not significantly different from the treatments performed with T, except in *S. fuscovarius* at 0.001 µM. Significant differences were found between the 0.001 and 1 µM doses in follicles of the three species treated with both hormones ($P < 0.05$).

3.2 | Maturation dynamics induced by P_4 and T

Maturation dynamics was approached from an experimental and modeling viewpoint. Based on the results obtained from dose–response curves (Figure 1), the concentration of P_4 and T chose to perform time–response curves was 1 µM. The follicles of *S. fuscovarius*, *P. borellii*, and *P. biligonigerus* were incubated for variable times up to 24 hr. After incubation GVBD percentages were controlled. The experimental results obtained were used for modeling and simulation.

The Gompertz mathematical model of growth was used to explain the behavioral dynamics of the oocyte maturation process in experimental conditions. Experimental data and the solution curve of the simulation are shown in Figure 2a–c.

Experimental data obtained in *S. fuscovarius* in response to P_4 (Figure 2a) show that after 2 hr of incubation there was a rapid increase in GVBD percentages. Fifty percent maturation was reached between hours 3 and 4. Around 100% GVBD was observed after 12 hr of treatment. However, the experimental data obtained in follicles treated with T indicate that the biological response was slower, starting at 4 hr and reaching a maximum percentage of GVBD at 9 hr, which was higher than the response of follicles treated with P_4 for the same time periods.

Similar behavior was observed in *P. borellii* follicles (Figure 2b), where maturation induced with T started later than with P_4. In this species, GVBD percentages obtained with T were kept below those obtained with P_4 up to 21 hr, at which time the follicles responded in a similar way to both steroids.

In contrast to the data obtained for *S. fuscovarius* and *P. borellii*, in *P. biligonigerus* (Figure 2c) a similar response was observed when follicles were treated with P_4 and T. However, after 5 hr, the response to P_4 slightly exceeded that of T, this trend being maintained up to 24 hr.

In *S. fuscovarius* and *P. borellii* the response to P_4 occurred earlier than in follicles treated with T. However, in *P. biligonigerus*, these two hormones showed very similar dynamics.

The solution trajectory of the Gompertz model (Figure 2a–c) shows the behavior of GVBD dynamics. Once GVBD started, rapid growth was observed at initial times and became slower at the end of the process. Growth was linear around time τ defined in Equation (7).

The solution trajectories in the three species were suitably adjusted to the corresponding experimental data, validating the Gompertz model with the respective values of r and K parameters presented in Table 1.

The measurement of GVBD dynamics is given by the values of parameter r in Table 1, which are indicators of the speed with which GVBD occurred between the species studied and between steroids. In *P. biligonigerus*, the values of r for both steroids are similar. In the case of *P. borellii*, the value of r for P_4 ($r = 1.184$) indicates that the biological response to this hormone was faster than with T ($r = 0.773$). However, in *S. fuscovarius* follicle maturation in the presence of T was faster, in agreement with the value of the r parameter ($r = 1.800$) in Table 1.

In relation to the K parameter, in all species tested the model slightly underestimates the value of K_{exp} for both steroids.

The inflection level $x_\tau = (K/e) \leq (36.78\%)$ represents the percentage of oocytes that mature at the inflection time $\tau = (\ln \beta/r)$ given in Equation (7). The inflection points (τ, x_τ) for each species were estimated according to K, r, and β parameters. Time τ differs according to the hormone and the species studied (Table 1) and was reached between two key moments of maturation: the resumption of meiosis and metaphase II.

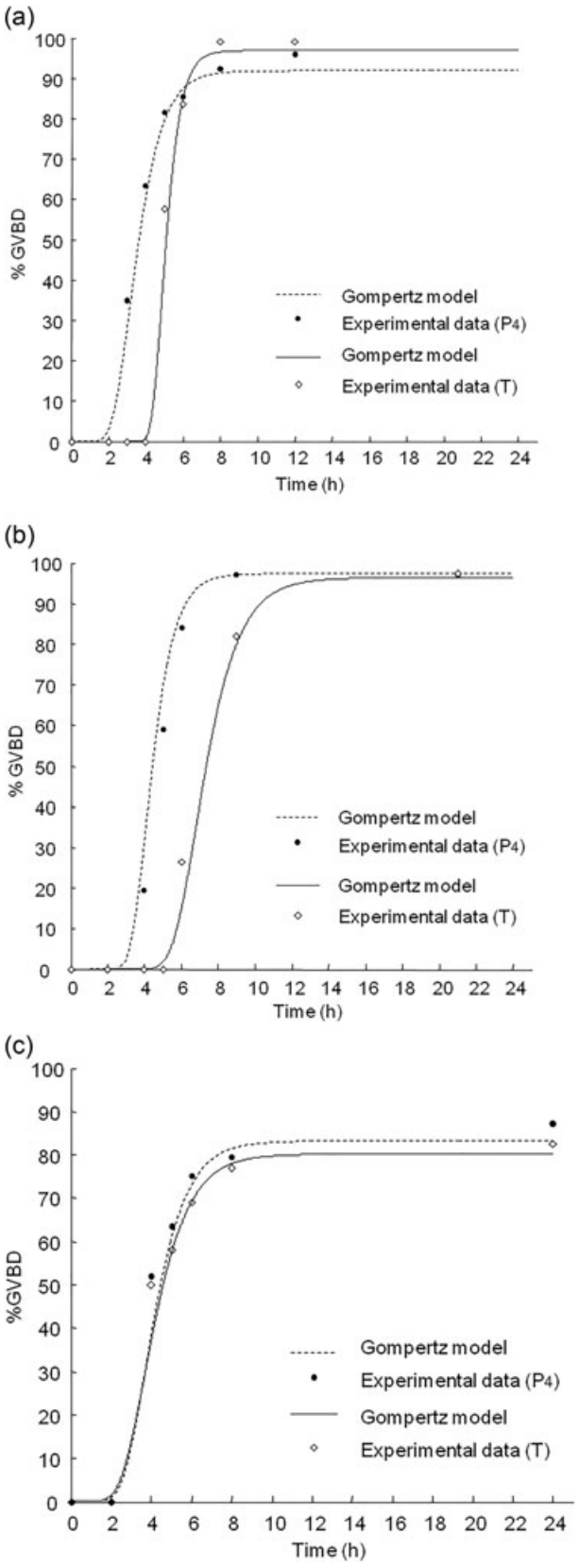

Figure 2a–c shows that the model presents an initial lag phase that corresponds to the absence of evident morphological changes in follicles treated with P_4 and T. In *S. fuscovarius* and *P. borellii*, the duration of the lag phase differed according to the hormone tested and varied between 0 and 4 hr (Figure 2a). In contrast, in *P. biligonigerus* the duration of the lag phase for P_4 and T was the same.

The K parameter in the model represents the maximum percentage of oocytes that broke the germinal vesicle. The value of K varied according to the species and hormone tested, reaching a minimum value (80.3%) for the follicles of *P. biligonigerus* treated with T and a maximum value (97.28%) for the follicles of *P. borellii* treated with P_4 (Table 1).

The simulation allowed the obtainment of time t_{II} corresponding to the moment from which the K level was reached in each species (Table 1). Time t_{II} is associated with the moment at which meiosis is arrested in metaphase II. Table 1 shows that *S. fuscovarius* is the species that reached metaphase II earlier when follicles were treated with T, whereas *P. borellii* reached metaphase II later in time when follicles were treated with T. In *P. biligonigerus* no difference can be observed between treatment with T and P_4 and oocytes reached metaphase II neither too early nor too late (≈ 11hr). The point (t_{II}, K) belonging to the stationary phase represents the asymptotically stable equilibrium point of the Gompertz model.

4 | DISCUSSION

Results herein reported constitute the first study that addresses the ovarian physiology of three species of amphibians native to South America: *P. borellii*, *S. fuscovarius* and *P. biligonigerus* and that propose Gompertz mathematical model to explain the maturation dynamics of their oocytes. With an interdisciplinary approach, the behavior of the oocyte maturation process is discussed through experimentation, modeling, and corresponding simulation.

Experimentation showed that the steroids tested were able to induce GVBD similarly in oocytes of females of *P. borellii*, *S. fuscovarius*, and *P. biligonigerus* captured during the reproductive period.

FIGURE 2 Experimental data versus Gompertz model. Dots represent experimental data of time–response curves induced by P_4 and T in follicles of (a) *S. fuscovarius*, (b) *P. borellii*, and (c) *P. biligonigerus*. Follicles were incubated in AR and maturation was induced with P_4 (1 μM) and T (1 μM; $n = 3$). GVBD was examined every 2 hr. Results are presented as means for each treatment. Curves represent the solution trajectory of the Gompertz model for each experimental treatment. AR: Amphibian Ringer; GVBD: germinal vesicle breakdown; P_4: progesterone; T: testosterone

TABLE 1 Gompertz model parameters in response to P_4 and T in follicles of *Scinax fuscovarius*, *Pleurodema borellii* , and *Physalaemus biligonigerus*

Species	Hormone	r (hr^{-1})	K (%)	K_{exp} (%)	τ (hr)	x_τ (%)	t_{II} (hr)
S. fuscovarius	P_4	1.052	92.9	96	3.16	35.32	9.6
	T	1.800	97.6	99	4.87	36.42	8.6
P. borellii	P_4	1.184	97.28	97	4.1	36.60	11.8
	T	0.773	96.29	97.5	6.8	35.86	18.6
P. biligonigerus	P_4	0.889	83.3	87.2	3.72	32.09	11.3
	T	0.878	80.3	82.6	3.69	29.20	11.8

Note. K_{exp} represents the maximum experimental value of mature follicles. P_4: progesterone; T: testosterone.

No significant differences were found in dose–response curves between P_4 and T treatments. However, studies carried out in *R. arenarum* (Arias Torres, Buhler, & Zelarayán, 2016) suggest that P_4 (1 μM) is the most efficient inducer of in vitro maturation of oocytes in both the reproductive and nonreproductive periods, whereas Haccard et al. (2012) showed that follicles of *X. laevis* mainly synthesize dehydroepiandrosterone (DHEA) and that this androgen is able to induce GVBD in its oocytes in a similar way to T. In this species, when the ovaries are stimulated with human chorionic gonadotropin, T is the main maturation inducer (Lutz et al., 2001). Authors postulate that T is the physiological inducer of the maturation of *X. laevis* oocytes and that, during its synthesis in the ovary, the oocyte has active participation (Deng et al., 2009).

In *R. arenarum*, oocyte response to different androgens (T, androstenedione, and DHEA) throughout the year was variable but in no case greater than when induced by P_4.

During the reproductive period of *R. arenarum* ovarian steroidogenesis is directed towards P_4 synthesis. In the nonreproductive period, however, ovarian secretion of T increases to be converted into estradiol, a steroid that promotes follicular growth (Arias Torres, Páez, Unías, & Zelarayán, 2018).

Reproductive events of amphibians are closely involved to a variety of intrinsic (neuroendocrine processes) and extrinsic factors (environmental condition such as temperature, photoperiod, humidity, and precipitation; Vu & Trudeau, 2016). Given that the species analyzed in this paper share the same environment as *R. arenarum* and are under similar seasonal conditions, it is possible that, even though we found the effect of P_4 and T on maturation did not vary significantly, they all share common features in the ovarian physiology. Therefore, during the reproductive period and after follicles have reached the maximum development in the ovary, steroid synthesis is directed to P_4 production (Arias Torres, 2017). However, other tests will be necessary to confirm our results.

From the viewpoint of maturation dynamics, laboratory results on an experimental scale allow us to consider that T and P_4 resumed meiosis in treated follicles at variable times on each of the three amphibian species studied. In Figure 2a–c the experimental data show a sigmoidal behavior, which agrees with the Gompertz model curve proposed in this study. Three stages with different behaviors are well defined: initial latency phase (lag phase), exponential phase, and stationary phase. These phases correspond to different times and meiotic stages.

From a biological point of view, the lag phase is used to refer to the time that elapses between the application of a stimulus (the hormone) and the manifestation of the corresponding response. In particular, for this study, the lag phase would represent the period occurring since oocyte incubation with the hormone and in which the signaling pathways leading to meiotic resumption and consequently to GVBD are triggered. During this time gap oocytes do not show morphological signs of maturation.

When the lag phase is over, the Gompertz model curve presents a very fast growth phase known as the exponential phase, determined by the value of its parameter r, which is related to the speed with which the oocytes mature. Biologically, the exponential phase would be associated with the period between the reinitiation of meiosis and the different stages before reaching metaphase II.

Activation of the signaling cascades that lead to the morphological changes expressed as GVBD occurs during this phase in the oocyte. Different biochemical processes allow this phenomenon to occur. In fact, after the P_4 stimulus, in the oocyte arrested in the G2 phase, a cascade of MAP kinases is activated, and the oocyte leaves the G_2 arrest state to enter meiosis I and then reaches metaphase II, stage in which the cell cycle stops again (Ferrell & Xiong, 2001).

Finally, the curve of the model reaches the stationary phase, the time at which the highest GVBD percentage (K) occurs (Table 1). K represents the equilibrium level associated with the steady state, at which time changes will no longer occur, that is, $(dx/dt) = 0$ in Equation (1). Biologically, it would mean that GVBD has already happened in most oocytes and that metaphase II has been reached.

The dynamics of the different phases varies according to the amphibian species analyzed and the hormone inducing the maturation process. In *P. biligonigerus* Gompertz model curves (Figure 2c), the lag phase corresponds to a short time period ($0 < t < 2$), as does the response to P_4 in *S. fuscovarius* (Figure 2a), *P. borellii* (Figure 2b). In these last two species, T induces a slower response. In fact, the lag phase corresponds to a longer time period ($0 < t < 4$).

Results presented in this study show differences with respect to those obtained from the modeling of the ovarian maturation process

of *R. arenarum* (Benzal & Zelarayán, 2015), where the lag phase took up to 6 hr in follicles treated with P_4, whereas in the species here analyzed the lag phase does not exceed 4 hr with any of the steroids tested. Comparatively, in *R. arenarum* oocytes, maturation with P_4 ($r = 0.907$) occurred more rapidly than when induced with T ($r = 0.692$), similarly to *P. borellii*.

However, for *S. fuscovarius*, the *r* value obtained is higher for T ($r = 1.800$) than for P_4 ($r = 1.052$), whereas in *P. biligonigerus* the value of this parameter is equal for the two hormones.

In this study, the experimental data (Figure 2a–c) allows the visualization of the maturation process, reaching only qualitative conclusions. However, GVBD dynamics can be modeled to quantify the speed of the maturation process for the different steroids used in each amphibian species studied (Table 1).

In this study, the value of the *r* parameter quantifies the speed of the process and is related to the efficiency of the inducing hormone, which would be of interest in studies on biological processes after ovulation such as successful fertilization of the mature oocyte.

Likewise, the biological meaning is attributed to parameters *K* and β (Equations 1 and 2). The *K* parameter is associated with the maximum GVBD percentage reached (mature oocytes) and from a mathematical point of view it represents the level of equilibrium corresponding to the time at which the biological process reaches the steady state and would be associated with the moment when the chromosomes are arranged on the equatorial plate of metaphase II.

Quantification of τ time (Table 1) from the estimated β parameter has allowed validation of the proposed model, so that its solution trajectory simulates the evolution of the process under study. In particular, the quantification of the *r* parameter, associated with the speed of the maturation process, becomes important as it would be one of the parameters indicative of steroids efficiency in the process in relation to the dose used.

The biological meaning attributed to the parameters is the result of interdisciplinary work during the final phase of modeling and simulation. Similarly, parameters are meaningful in other studies on tumor growth (Menchón, 2007), microbial behavior (Cayre et al., 2001), and animal production (Alvarez et al., 2005; Casas et al., 2010), among others.

Modeling and simulation are meaningful as they allow us to infer the behavior of biological processes when experimental data are not available, as is the case in this process during the latency phase. It also allows for adjustments in the experimental design since the moment after which there will be no changes in the biological process can be inferred. An indicator of this state is the t_{II} time is corresponding to the steady state.

Mathematical models can be useful to understand the dynamics of systems and biological processes. It is clear that a model does not suppress experimental data, as these are the source of the model's richness and its validation. Research needs both experimentation and modeling to understand systems and processes as complex as those found in biology.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial assistance of The Secretaría de Ciencia, Arte e Innovación Tecnológica (SCAIT) of the Universidad Nacional de Tucumán.

ORCID

Carolina Manzano http://orcid.org/0000-0003-4166-8499

REFERENCES

Alvarez, M., Quintana, H., Mallo, G., & Quinn, M. E. (2005). Chinchilla (*Chinchilla lanigera*) body growth in a commercial production. *Archivos Latinoamericanos de Producción Animal*, *5*(3), 72.

Arias Torres, A. J. (2017). *Rol de los esteroides en la maduración de los ovocitos de Rhinella arenarum* (PhD thesis). Universidad Nacional de Tucumán, Tucumán, Argentina.

Arias Torres, A. J., Bühler, M. I., & Zelarayán, L. I. (2016). In vitro steroid-induced meiosis in *Rhinella arenarum* oocytes: Role of pre-MPF activation. *Zygote*, *24*(2), 252–258.

Arias Torres, A. J., Páez, J. B., Unías, L., & Zelarayán, L. I. (2017). New evidence of physiological role of progesterone on *R. arenarum* oocyte maturation. *Biocell*, *41*(Suppl) 1. A1–A141. A16.

Arias Torres, A. J., Páez, J. B., Unías, L., & Zelarayán, L. I. (2018). Seasonal changes in the synthesis of E2, P4 and T in the ovary of *Rhinella arenarum*. *Biocell*, *42*(Suppl) 2. ISSN 1667-5746, (online version) A50.

Benzal, M. G. (2003). *Modelización y simulación matemática de procesos de recuperación de metales de aguas residuales mediante membranas líquidas soportadas en configuración de fibra hueca* (PhD thesis). Universidad Politécnica de Cataluña, Barcelona, España.

Benzal, M. G., & Zelarayán, L. I. (2015). Math as a tool in biology: *Rhinella arenarum* oocyte maturation. *Biocell*, *39*(Suppl 5), A 65.

Bühler, M. I., Sánchez Toranzo, G., & Zelarayán, L. I. (2014). El ovocito de *Rhinella arenarum* como modelo para el estudio de la maduración y la activación. *Editorial Opera lilloana 48*, (pp. 1–172). Fundación M. Lillo. ISSN 950-668-010-8.

Casas, G. A., Rodriguez, D. A., & Téllez, G. A. (2010). Mathematical properties of the Gompertz model and its application to the growing pig. *Revista Colombiana de Ciencias Pecuarias*, *23*(3), 349–358.

Cayre, M., Vignolo, G. & Garro, O. (2001). Validación y comparación de modelos de crecimiento microbiano. Universidad Nacional Del Nordeste, Comunicaciones Científicas y Tecnológicas, Argentina.

Deng, J., Carbajal, L., Evaul, K., Rasar, M., Jamnongjit, M., & Hammes, S. R. (2009). Nongenomic steroid-triggered oocyte maturation: Of mice and frogs. *Steroids*, *74*, 595–601.

Ferrell, J. E., Jr., & Xiong, W. (2001). Bistability in cell signaling: How to make continuous processes discontinuous, and reversible processes irreversible. *Chaos*, *11*(1), 227–236.

Glynn, P., Unudurthi, S. D., & Hund, T. J. (2014). Mathematical modeling of physiological systems: An essential tool for discovery. *Life Sciences*, *111*(1), 1–5.

Haccard, O., Dupré, A., Liere, P., Pianos, A., Eychenne, B., Jessus, C., & Ozon, R. (2012). Naturally occurring steroids in *Xenopus* oocyte during meiotic maturation. Unexpected presence and role of steroid sulfates. *Molecular and Cellular Endocrinology*, *362*(1), 110–119.

Karkach, A. (2006). Trajectories and models of individual growth. *Demographic research*, *15*, 347–400.

Lutz, L. B., Cole, L. M., Gupta, M. K., Kwist, K. W., Auchus, R. J., & Hammes, S. R. (2001). Evidence that androgens are the primary steroids produced by *Xenopus laevis* ovaries and may signal through the classical androgen receptor to promote oocyte maturation.

Proceedings of the National Academy of Sciences, *98*(24), 13728–13733.

Maller, J. L. (2001). The elusive progesterone receptor in *Xenopus* oocytes. *Proceedings of the National Academy of Sciences*, *98*(1), 8–10.

Marteil, G., Richard-Parpaillon, L., & Kubiak, J. Z. (2009). Role of oocyte quality in meiotic maturation and embryonic development. *Reproductive biology*, *9*(3), 203–224.

Medina, M. F., Ramos, I., Crespo, C. A., González-Calvar, S., & Fernández, S. N. (2004). Changes in serum sex steroid levels throughout the reproductive cycle of *Bufo arenarum* females. *General and Comparative Endocrinology*, *136*(2), 143–151.

Menchón, S. (2007). *Modelado de las diversas etapas del crecimiento del cáncer y de algunas terapias antitumorales* (PhD thesis). Universidad Nacional de Córdoba, Córdoba, Argentina.

Nobile, A. G., Ricciardi, L. M., & Sacerdote, L. (1982). On Gompertz growth model and related difference equations. *Biological Cybernetics*, *42*(3), 221–229.

Norris, D. O., & Lopez, K. H. (2011). The endocrinology of the mammalian ovary. In D. O. Norris, K. H. Lopez (Eds.), *Hormones and reproduction of vertebrates* (pp. 59–72). Amsterdam: Elsevier.

Rasar, M. A., & Hammes, S. R. (2006). The physiology of the *Xenopus laevis* ovary. *Methods in Molecular Biology*, *322*(2006), 17–30.

SAS Institute Inc. (2004). SAS/STAT® 9.1 User's Guide. Cary, NC: SAS Institute Inc.

Sen, A., Prizant, H., & Hammes, S. R. (2011). Understanding extranuclear (nongenomic) androgen signaling: What a frog oocyte can tell us about human biology. *Steroids*, *76*, 822–828.

Smith, L. D. (1989). The induction of oocyte maturation: Transmembrane signaling events and regulation of the cell cycle. *Development*, *107*(4), 685–699.

The MathWorks Inc. (2008). *MATLAB version 7.6.0.*. Natick, MA: Author.

Vu, M., & Trudeau, V. L. (2016). Neuroendocrine control of spawning in amphibians and its practical applications. *General and Comparative Endocrinology*, *234*, 28–39.

Zelarayán, L. I., Ajmat, M. T., Bonilla, F., & Bühler, M. I. (2013). Involvement of G protein and purines in *Rhinella arenarum* oocyte maturation. *Zygote*, *21*(3), 221–230.

Zelarayán, L. I., Oterino, J., & Bühler, M. I. (1995). Spontaneus maturation in *Bufo arenarum* oocytes: Follicle wall involvement, respiratory activity, and seasonal influences. *Journal of Experimental Zoology*, *272*(5), 356–362.

How to cite this article: Manzano C, Benzal MG, Zelarayán LI. Dynamics of steroid-induced oocyte maturation in three amphibian species: Mathematical modeling and simulation. *J. Exp. Zool*. 2018;1–8. https://doi.org/10.1002/jez.2222