Zygote **9** (November), pp 353–359. © 2001 Cambridge University Press Printed in the United Kingdom

Nuclear maturation inducers in *Bufo arenarum* oocytes

Inés Ramos, Susana Cisint, Claudia Crespo, Marcela F. Medina and Silvia Fernández
Department of Developmental Biology, National Council for Scientific and Technical Research, National University of Tucumán, Chacabuco 461, (4.000) Tucumán, Argentina

Date submitted: 22.3.01. Date accepted: 15.5.01

Summary

The present study analyses the effect of dihydrotestosterone (DHT) and mammalian insulin on the nuclear maturation of *Bufo arenarum* oocytes under *in vitro* conditions. The response of fully grown follicle oocytes to DHT, shown by germinal vesicle breakdown (GVBD), occurred in a manner dependent on dose, time and sexual cycle period. The highest oocyte sensitivity to the hormone appeared during the breeding period, a fact evinced by high GVBD percentages after short incubation periods and at a low hormone concentrations. Insulin also proved effective in inducing nuclear maturation, although its action was only visible at high concentrations and after a long incubation period. The combination of insulin and steroid hormones (DHT or progesterone), both at subliminal doses, caused a noticeable potentiating synergism, resulting in a rapid and important increase in GVBD. Another effect of insulin was the acquisition by oocytes of steroid sensitivity during folliculogenesis.

Keywords: Amphibian, Dihydrotestosterone, Insulin, Nuclear maturation, Oocyte

Introduction

In the reproductive cycle of *Bufo arenarum* females three stages are clearly identifiable: a post-ovulatory or gonadal recovery period lasting from December to April, a quiescent or hibernation period from May to July–August, and a reproductive period from September to November (Valdez Toledo & Pisanó, 1980).

During the gonadal recovery and quiescent periods follicle growth and differentiation (folliculogenesis) occur. These two processes provide an appropriate microenvironment for the growth and development of the oocytes through the different stages of oogenesis as well as for the later acquisition of their maturation capacity. Throughout the intraovarian growth phase *Bufo arenarum* oocytes, like those of other vertebrates, remain arrested at the prophase of the first meiotic division (Masui, 1985; Nagahama, 1994).

During the breeding period and the later stages of follicle development the oocytes that exhibit a maximum degree of development (fully grown oocytes) are meiotically competent, that is, gametes are capable of reinitiating and completing the first meiotic division (Liu & Patiño, 1993; Downs, 1997). This process, known as nuclear maturation, involves the migration of the nucleus or germinal vesicle (GV) towards the surface of the animal pole (Wasserman *et al.*, 1986) and the later nuclear envelope breakdown (Wasserman *et al*, 1986; Ramos *et al.*, 1998). At the same time, a series of biochemical, functional and structural changes (Waserman *et al.*, 1988; Lessman & Kessel, 1992, Ramos *et al.*, 1999) take place at the cytoplasmic level (cytoplasmic maturation). The two processes culminate in ovulation, thus providing a gamete competent for fertilisation and normal development.

Meiotic resumption, known to be hormonally regulated, involves a functional hypothalamus–hypophysis–gonad axis. In *Bufo arenarum*, hypothalamic neurones have been proved to secrete a gonadotropin-releasing hormone (GnRH), similar to that of mammals (Miranda *et al.*, 1998), which regulates secretion at the hypophysis level of two hormones: a follicle-stimulating hormone (FSH) and a luteinising hormone (LH) with biochemical and immunological properties similar to those of the gonadotropins of other tetrapods (Licht *et al.*, 1983). FSH and LH are both involved in the regulation of ovarian steroid secretion (Itoh & Ishii, 1990; Polzonetti-Magni *et al.*, 1998).

During the reproductive period, immediately before the onset of the maturation process, follicle cells increase the synthesis and secretion of progesterone in response to the action of gonadotropins (Maller & Krebs, 1980). At the same time, an increase in the

All correspondence to: Inés Ramos, Chacabuco 461, 4000 – S.M. de Tucumán, Argentina. Fax: +543 81 424805. e-mail: inramos@unt.edu.ar

expression of steroid receptors at the level of the oocyte plasma membrane can be observed (Maller & Krebs, 1980; Sadler & Maller, 1982; Liu & Patiño, 1993). By means of its interaction with these specific binding sites, progesterone induces nuclear maturation (Maller & Krebs, 1980; Jalabert *et al*., 1991) by triggering a series of events that comprise the decrease in cAMP levels through the inhibition of the activity of adenylate cyclase (Sadler & Maller, 1985), the diminution of the cytostatic factor and the increase in the maturation-promoting factor (MPF) (Bar-Ami *et al*., 1994). Although progesterone is considered to be the main inducer of nuclear maturation in amphibians (Jalabert *et al*., 1991), this event can also be triggered by other hormones (Tonetta & DiZerega, 1989).

As regards steroid hormones, the presence of circulating testosterone and dihydrotestosterone (DHT), with high levels that fluctuate like those of estradiol during the reproductive cycle, has been demonstrated in the females of some lower vertebrates (Licht *et al*., 1983; Fernández *et al*., 1984; Nagler & Idler, 1992). In fish both testosterone and estradiol exhibit a positive correlation with the gonadosomatic index (Nagler & Idler, 1992), while in *Rana esculenta* this correlation occurs only with androgens (Licht *et al*., 1983). Studies carried out in our laboratory have shown that the level of these androgens in *Bufo arenarum* serum is higher than that of estradiol (Fernández *et al*., 1984). The biological significance of the high levels of testosterone and DHT found in the females of these species has not been elucidated.

Among peptide compounds, insulin is one of the hormones capable of regulating ovarian function by affecting the differentiation and development of granulosa cells, reducing the number of atretic follicles, and modulating steroidogenesis by increasing aromatase activity (Matamoros *et al*., 1990). As regards the action of insulin on oocytes, there exist contradictory reports concerning its effect on nuclear maturation. Studies carried out in oocytes from *Xenopus* (El-Etr *et al*., 1979) and *Rana pipiens* (Lessman & Schuetz, 1981) have proved that insulin can induce meiotic reinitiation by acting on specific receptors at the membrane level. However, in *Xenopus* denuded oocytes the data obtained indicate that insulin by itself does not affect nuclear maturation (Hirai *et al*., 1983; Le Goascogne *et al*., 1985).

The present study aimed to analyse the role of DHT and insulin in the control of nuclear maturation in *Bufo arenarum* oocytes as well as the effect of the latter in combination with DHT or progesterone.

Materials and methods

Animals

Sexually mature *Bufo arenarum* female toads were freshly collected in the neighbourhood of San Miguel de Tucumán, Argentina, during two stages of the sexual cycle: the quiescent period (May–August) and the breeding period (September–November). Animals were used immediately after capture or maintained for brief periods in boxes with appropriate humidity at room temperature until use.

Nuclear maturation induction

Fully grown follicle oocytes (1.5–1.7 mm in diameter) with one layer of follicle cells were dissected from ovarian tissue with watchmaker's forceps under a stereo dissecting microscope and placed in amphibian Ringer's solution. They were then pooled at random in batches of about 150 oocytes, each in a Petri dish containing 30 ml of Ringer's solution supplemented with penicillin G sodium (30 mg/l) and streptomycin sulphate (50 mg/l) as incubation medium.

Nuclear maturation was induced by incubating batches of follicles for up to 24 h in the presence of DHT or progesterone (Sigma Chemical Co.), both dissolved in ethanol at a ratio of 1 mg/ml, and insulin (porcine, Sigma) containing 0.5% zinc dissolved in Ringer's solution. Treatments were performed at 25 ± 1 °C. Control follicles were incubated under the same conditions but without the hormones.

Nuclear maturation response was scored at intervals of 2 or 4 h during the treatment period and expressed as the percentage of germinal vesicle breakdown (GVBD). Oocyte maturation was verified by dissecting follicles after fixation for 48 h in Ancel and Vintemberger.

Experimental data are expressed as the mean ± SEM of the number of duplicate incubations specified in each legend. Statistical analysis of values was undertaken by Student's *t*-test, $p < 0.05$ being considered statistically significant.

Results

Effect of DHT

Previous results obtained in our laboratory suggested that oocyte sensitivity to nuclear maturation in response to DHT was dependent on the period of the sexual cycle of *Bufo arenarum* females. This fact led us to study the effect of DHT at two stages of the cycle: the quiescent and the breeding period of the species.

When oocytes surrounded by one layer of follicle cells, obtained from the ovaries of animals captured

https://doi.org/10.1017/S0967199401001411 Published online by Cambridge University Press

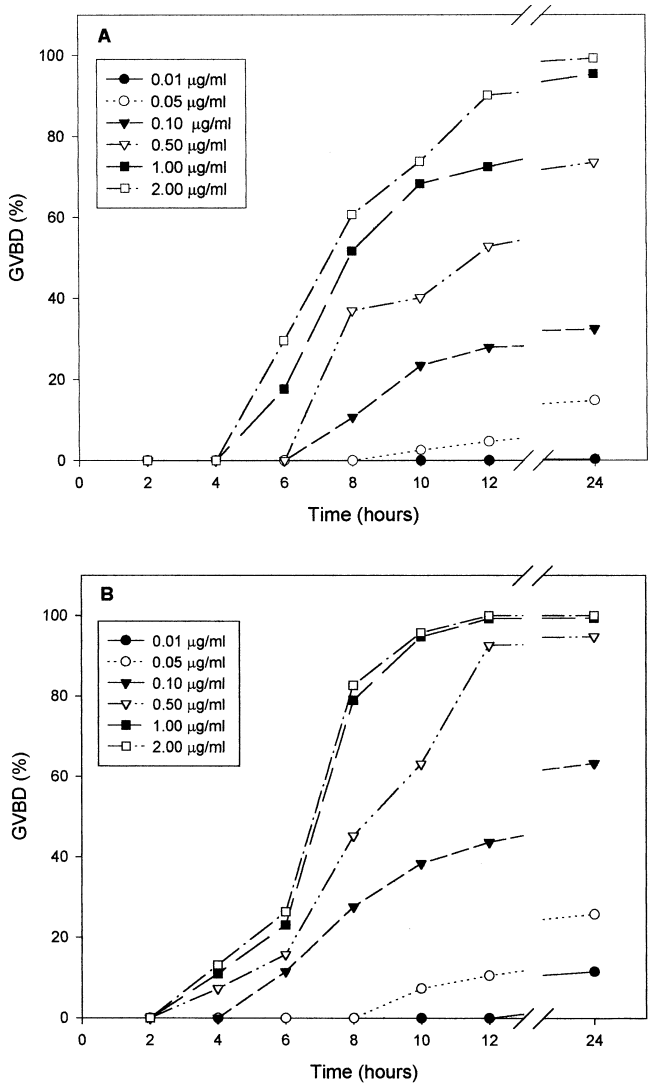

Figure 1 Dose and time course of germinal vesicle breakdown (GVBD) induced by dihydrotestosterone (DHT). Follicles in batches of 150 were incubated at 25 ± 1 °C in 30 ml of Ringer's solution plus DHT at different concentrations. About 15 oocytes were scored each time. Results are shown as the average from experiments performed with different females. (*A*) Follicles obtained from animals captured during the quiescent period ($n = 7$). (*B*) Follicles obtained from animals captured during the breeding period ($n = 9$).

during the quiescent period, were incubated in the presence of increasing amounts of DHT (0.01–2 µg/ml), the lowest dose (0.01 µg/ml) was seen to have no effect on nuclear maturation (Fig. 1*A*).

The action of the hormone became evident at low percentages (15–30% GVBD) after treatment for 24 h with a dose of 0.05 and 0.1 µg/ml respectively. A significant response to the hormone (65% GVBD) was obtained with a dose of 0.5 µg/ml after the same incubation period, and maximum GVBD percentages (98–100%) were attained only with the highest doses assayed after treatment for 24 h ($p < 0.01$).

On analysing the response of the follicles obtained from females captured during the breeding period and incubated in the same conditions we observed 60% GVBD after treatment for 24 h with a dose of 0.1 µg/ml (Fig. 1*B*). From a 0.5 µg/ml concentration onwards, 95–100% GVBD was attained after the same incubation period ($p < 0.01$).

It is important to note that, in contrast to the results obtained during the quiescent period, during the reproductive period a remarkable response to the hormone (about 85% GVBD) was observed with doses ranging from 1 to 2 µg/ml between 8 and 10 h after incubation.

Effect of insulin

To analyse the effect of insulin on meiotic reinitiation, batches of follicles isolated from animals captured during the breeding period were incubated in the presence of the hormone at doses ranging between 10 and 80 µg/ml. Nuclear maturation was scored at different times during a 24 h period.

The results shown in Table 1 demonstrate that insulin is not very effective at inducing meiotic resumption. Only with the highest doses assayed (60 and 80 µg/ml) and after treatment for 24 h could a significant GVBD percentage be obtained (40–56%).

When batches of follicles were preincubated for 1 h with subliminal insulin concentrations (10 and 20 µg/ml) and later treated for 24 h with the minimum dose of DHT used in our experiments (0.01 µg/ml), a significant increase (potentiating synergism) could be observed in the percentages of GVBD ($p < 0.01$) compared with the values obtained through the action of the steroid alone (Fig. 2*A*). A similar response occurred in parallel experiments by incubating the follicles with progesterone after pretreatment for 1 h with insulin, both at subliminal concentrations (Fig. 2*B*).

It is important to note that this potentiation effect, obtained by the combination of the peptide hormone with each of the steroids assayed, became visible only after treatment for 8 h and underwent a progressive increase during the experimental period (Fig. 2*A*, *B*).

Table 1 Effect of insulin on nuclear maturation (scored as the percentage of germinal vesicle breakdown)

Incubation time (h)	Insulin (µg/ml)					
	0	10	20	40	60	80
4	0	0	0	0	0	0
8	0	0	0	0	0	5
12	0	0	0	0	10	18
24	0	3	13	15	40	56

Values represent the median of data obtained by experiments performed with different animals (n=6).

https://doi.org/10.1017/S0967199401001411 Published online by Cambridge University Press

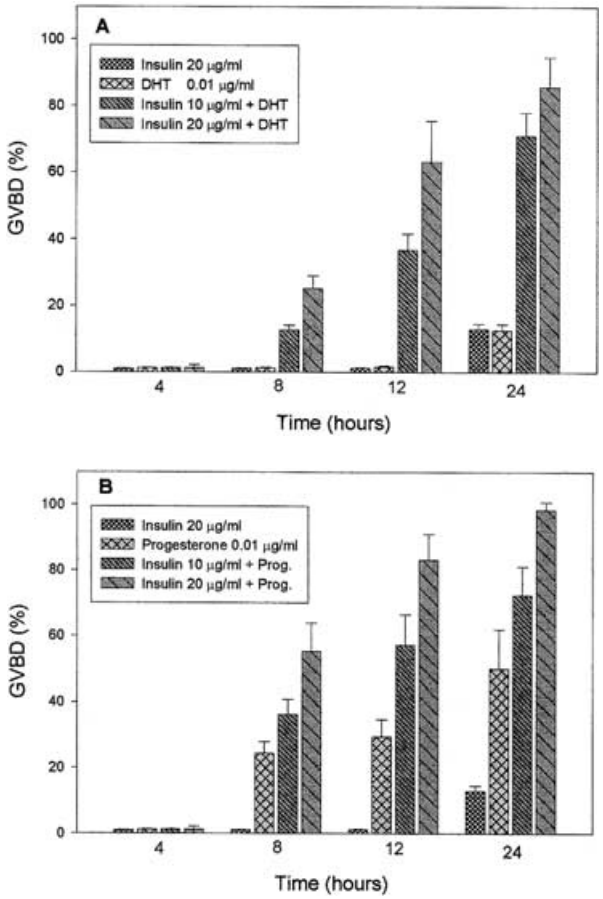

Figure 2 Potentiating effect of insulin on dihydrotestosterone (DHT)- or progesterone-induced GVBD.
Follicles were preincubated for 1 h with insulin before inducing nuclear maturation by addition of DHT or progestrone to the incubation medium. (*A*) Insulin plus DHT. (*B*) Insulin plus progesterone. Data represent the mean ± SEM of experiments carried out with different animals ($n = 6$).

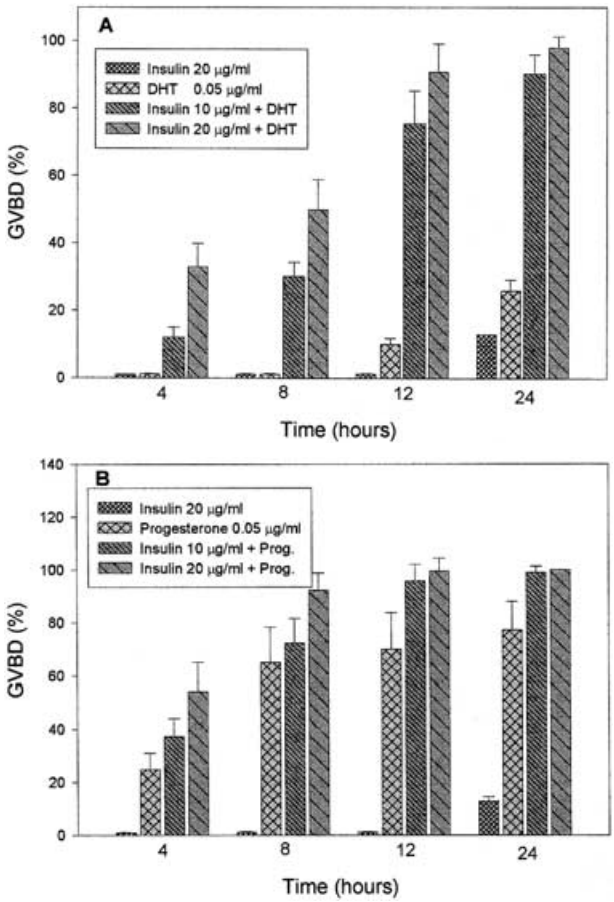

Figure 3 Potentiating effect of insulin with higher doses of dihydrotestosterone (DHT) or progesterone on germinal vesicle breakdown (GVBD). Follicles were treated as indicated in Fig. 2. (*A*) Insulin plus DHT. (*B*) Insulin plus progestrone. Data represent the mean ± SEM of experiments performed with different animals ($n = 6$).

When insulin combined with a higher dose of DHT or progesterone (0.05 µg/ml), two noticeable events could be observed (Fig. 3*A*, *B*): a faster response than the one obtained in the above case, demonstrated by the high GVBD percentages after treatment for only 4 h, and a weaker effect obtained with the combination of peptide and steroid hormones with respect to those observed with the steroids alone.

To determine the stage of gonadal development at which the above effect of insulin appeared, batches of follicles containing oocytes at different stages of oogenesis (previtellogenic, early vitellogenic, late vitellogenic, auxocytosis and fully grown) (Valdez Toledo & Pisanó, 1980) were incubated with insulin for 1 h. Later, progesterone was added to the medium at a dose previously determined as highly effective (1 µg/ml) for inducing nuclear maturation in fully grown follicle oocytes (de Romero *et al.*, 1998). Control lots were incubated with progesterone alone, all treatments being carried out at 25 ± 1 °C for 24 h after progesterone addition.

The results showed that treatment either with progesterone alone or with progesterone plus insulin did not affect the germinal vesicle of the oocytes during the first two stages under study (data not shown). During the late vitellogenic stage (Table 2), although progesterone per se failed to induce GVBD, a potentiating effect was obtained by associating it with insulin. This effect became evident after incubation for 12 h, reaching significant values at 24 h ($p < 0.01$). It must also be noted that, even though progesterone by itself induced nuclear maturation in oocytes in the auxocytosis stage, the response rate was higher in the presence of insulin. Similar results (not shown) were obtained with DHT.

https://doi.org/10.1017/S0967199401001411 Published online by Cambridge University Press

Table 2 Effect of the combination of insulin and progesterone on oocyte nuclear maturation (scored as the percentage germinal vesicle breakdown) at different folliculogenic stages following incubation for 4, 8, 12 or 24 h

Oocyte stage	Progesterone (1 µg/ml)				Insulin (20 µg/ml) + progesterone (1 µg/ml)			
	4 h	8 h	12 h	24 h	4 h	8 h	12 h	24h
Late vitellogenic	0	0	0	0	0	0	40	80
Auxocytosis	0	75	100	100	57	100	100	100
Fully grown	28	97	100	100	85	100	100	100

Values represent the median of data obtained by experiments performed with different animals (n=6).

Discussion

Even though progesterone is considered to be the hormone that physiologically triggers meiotic resumption in amphibians (Jalabert *et al*., 1991), our observations demonstrate that DHT, an androgen with extremely high circulating levels in *Bufo arenarum* females (Fernández *et al*., 1984), is also capable of inducing nuclear maturation, its action being dose- and time-dependent.

The present study demonstrates that oocyte sensitivity to DHT varies according to the period of the sexual cycle of *Bufo arenarum* females. Comparison of the results obtained during the two periods under study, the quiescent and the breeding period, shows that during the latter oocytes become more sensitive to DHT action, as demonstrated by the larger percentages of GVBD obtained after shorter incubation times and at lower concentrations of the steroid.

These variations in the sensitivity to the hormone might be related to fluctuations in the circulating levels of DHT during the sexual cycle of *Bufo arenarum* females (unpublished results). In fact, the highest levels of the androgen were detected towards the end of the quiescent period and the start of the preovulatory stage. It should be noted that in *Scaphiopus couchii* an increase in DHT levels has been demonstrated during the period immediately before mating (Harvey *et al.*, 1997), a stage in which nuclear maturation and then ovulation would take place.

Another possible reason for the variation in oocyte sensitivity could be that, during the reproductive period, the fully grown oocyte might be physiologically affected by various hormones, the concerted action of which would allow it to acquire the competence to respond not only to progesterone but also to other inducers such as DHT.

In the case of certain mammalian (Totey *at al*., 1996) and amphibian (El Etr *et al*., 1979; Lessman & Marshall, 1984) species it has been demonstrated that ovarian function is regulated not only by gonadotropins but also by the concerted action of other peptide hormones such as insulin. This hormone plays an important role in the physiology of gonads, favouring the response of the ovary to gonadotropins and stimulating steroidogenesis (Srivastava & van der Kraak, 1994).

Our results indicate that insulin *per se* is effective in inducing nuclear maturation only at high concentrations and after long incubation periods. This observation agrees with the findings for *Xenopus* oocytes, and might be explained either because insulin activity in these species is mediated by low-affinity receptors (Maller & Koontz, 1981) or because these incubation conditions are necessary for insulin to effectively induce progesterone release by follicular cells. The latter possibility would also explain why denuded *Xenopus* oocytes fail to respond to treatment with insulin alone (Le Goascogne *et al*., 1985).

When we administered subliminal insulin doses together with DHT or progesterone in our experimental system, high percentages of GVBD were obtained. Similar results were described for denuded *Xenopus* oocytes (Le Goascogne *et al*., 1987), a fact that suggests that the synergistic effect of the two hormones is independent of the presence of follicle cells.

Our data demonstrate that incubation for a short period with low insulin doses produces a marked potentiation effect in the presence of steroid hormones (DHT or progesterone). The synergistic effect was less obvious when the steroid concentrations were increased, probably because larger doses are increasingly effective in inducing GVBD by themselves.

The action of insulin in facilitating GVBD at low concentrations of DHT or progesterone is of great physiological interest.

It is important to note that the combination of DHT or progesterone with insulin was also effective in inducing GVBD in oocytes in the late vitellogenic stage, a period during which the steroids have no effect by themselves. These results suggest that, although insulin potentiates the effect of progesterone from the auxocytosis stage onwards, at a previous stage in gonadal development the peptide hormone causes, by mechanisms as yet unknown, the acquisition by oocytes of the capacity to respond to DHT or progesterone.

The results obtained in *Bufo arenarum* oocytes suggest that insulin, as regards DHT and progesterone, would cause potentiation synergism as well as the capacity to induce oocyte sensitivity to the maturation-inducing steroids.

https://doi.org/10.1017/S0967199401001411 Published online by Cambridge University Press

Acknowledgements

This work was supported by a grant from CIUNT, Consejo de Investigaciones de la Universidad Nacional de Tucumán, Argentina.

References

Bar-Ami, S., Zlotkin, E., Brandes, J.M. & Itskovitz-Eldor, J. (1994). Failure of meiotic competence in human oocytes. *Biol. Reprod*. **50**, 1100–7.

Downs, S.M. (1997). Involvement of purine nucleotide synthetic pathways in gonadotropin-induced meiotic maturation in mouse cumulus cell-enclosed oocytes. *Mol. Reprod. Dev*. **46**, 155–67.

El-Etr, M., Schorderet-Slatkine, S. & Baulieu, E.-E. (1979). Meiotic maturation in *Xenopus* laevis oocytes initiated by insulin. *Science* **205**,1397–9.

Fernández, S.N., Mansilla, C. & Miceli, D.C. (1984). Hormonal regulation of an oviducal protein involved in *Bufo arenarum* fertilization. *Comp. Biochem. Physiol*. **78**, 147–52.

Harvey, L.A., Propper, C.R., Woodley, S.K. & Moore, M.C. (1997). Reproductive endocrinology of the explosively breeding desert spadefoot toad, *Scaphiopus couchii. Gen. Comp. Endocrinol*. **105**, 102–13.

Hirai, S., Le Goascogne, C. & Baulieu, E.-E. (1983). Induction of germinal vesicle breakdown in *Xenopus laevis* oocytes: response of denuded oocytes to progesterone and insulin. *Dev. Biol*. **100**, 214–21.

Itoh, M. & Ishii, S. (1990). Changes in plasma levels of gonadotropins and sex steroids in the toad, *Bufo japonicus*, in association with behavior during the breeding season. *Gen. Comp. Endocrinol*. **80**, 451–64.

Jalabert, B., Fostier, A., Breton, B. & Weil, C. (1991). Oocyte maturation in vertebrates. In *Vertebrate Endocrinology. Fundamentals and Biomedical Implications*, vol. 4a (ed. P.K.T. Pang & M.P. Schreibman), pp. 23–90. San Diego: Academic Press.

Le Goascogne, C., Sananès, N., Gouézou, M. & Baulieu, E.-E. (1985). Testosterone-induced meiotic maturation of *Xenopus laevis* oocytes: evidence for an early effect in the synergistic action of insulin. *Dev. Biol*. **109**, 9–14.

Le Goascogne, C., Sananès, N., Gouézou, M. & Baulieu, E.-E. (1987). Alkaline phosphatase activity in the membrane of *Xenopus laevis* oocytes: effects of steroids, insulin and inhibitors during meiosis reinitiation. *Dev. Biol*. **119**, 511–19.

Lessman, C.A. & Kessel, R.G. (1992). Effects of acrylamide on germinal vesicle migration and dissolution in oocytes of the frog, *Rana pipiens. Exp. Cell Res*. **202**, 151–60.

Lessman, C.A. & Marshall, W.S. (1984). Electrophysiology of *in vitro* insulin- and progesterone-induced reinitiation of oocyte meiosis in *Rana pipiens. J. Exp. Zool*. **231**, 257–66.

Lessman, C.A. & Schuetz, A.W. (1981). Role of follicle wall in meiosis reinitiation induced by insulin in *Rana pipiens oocytes, Am. J. Physiol*. **241**, E51–6.

Licht, P., McCreery, B.R., Bames, R. & Pang, R. (1983). Seasonal and stress related changes in plasma gonadotropins, sex steroids, and corticosterone in the bullfrog, *Rana catesbeiana. Gen. Comp. Endocrinol*. **50**, 124–45.

Liu, Z. & Patiño, R. (1993). High-affinity binding of progesterone to the plasma membrane of *Xenopus* oocytes: characteristics of binding and hormonal and developmental control. *Biol. Reprod*. **49**, 980–8,

Maller, J.L. & Krebs, E.G. (1980). Regulation of oocyte maturation. *Curr. Topics Cell Regul*. **16**, 271–311.

Maller, J.L. & Koontz, J.W. (1981). A study of the induction of cell division in amphibian oocytes by insulin. *Dev. Biol*. **85**, 309–16.

Masui, Y. (1985). Meiotic arrest in animal oocytes. In *Biology of Fertilization*, vol. 1 (ed. C.B. Metz & A. Monro), pp. 189–219. Orlando: Academic Press.

Matamoros, I.A., Cox, N.M. & Moore, A.B. (1990). Exogenous insulin and additional energy affect follicular distribution, follicular steroid concentrations, and granulosa cell human chorionic gonadotropin binding in swine. *Biol. Reprod*. **43**, 1–7.

Miranda, L.A., Paz, D.A., Affanni, J.M. & Somoza, G.M. (1998). Identification and neuroanatomical distribution of immunoreactivity for mammalian gonadotropin-releasing hormone (mGnRH) in the brain and neural hypophyseal lobe of the toad *Bufo arenarum. Cell Tissue Res*. **293**, 419–25.

Nagahama, Y. (1994). Endocrine regulation of gametogenesis in fish. *Int. J. Dev. Biol*. **38**, 217–29.

Nagler, J.J. & Idler, D.R. (1992). *In vitro* ovarian estradiol-17β and testosterone responses to pituitary extract and corresponding serum levels during the prespawning to vitellogenic phases of the reproductive cycle in winter flounder *(Pseudopleuronectes americanus). Comp. Biochem. Physiol*. **101**, 69–75.

Polzonetti-Magni, A.M., Mosconi, G., Carnevali, O., Yamamoto, K., Hanaoka, Y. & Kikuyama, S. (1998). Gonadotropins and reproductive function in the anuran amphibian, *Rana esculenta. Biol. Reprod*. **58**, 88–93.

de Romero, I.R., de Atenor, M.B. & Legname, A.H. (1998). Nuclear maturation inhibitors in *Bufo arenarum* oocytes. *Biocell* **22**, 27–34.

Ramos, I., Cisint, S., Alcaide, M. & Campos Casal, F. (1998). Morphological and cytochemical changes during nuclear maturation in *Bufo arenarum* oocytes. *Biocell* **22**, 167–75.

Ramos, I., Winik, B., Cisint, S., Crespo, C., Medina, M. & Fernández, S. (1999). Ultrastructural changes during nuclear maturation in *Bufo arenarum* oocytes. *Zygote* **7**, 261–9.

Sadler, S.E. & Maller, J.L. (1982). Identification of a steroid receptor on the surface of *Xenopus* oocytes by photoaffinity labeling. *J. Biol. Chem*. **257**, 355–61.

Sadler, S.E. & Maller, J.L. (1985). Inhibition of *Xenopus* oocyte adenylate cyclase by progesterone: a novel mechanism of action. *Adv. Protein. Phosph. Res*. **19**, 179–94.

Srivastava, R.K. & Van der Kraak, G. (1994). Insulin as an amplifier of gonadotropin action on steroid production: mechanisms and sites of action in goldfish prematurational full-grown ovarian follicles. *Gen. Comp. Endocrinol*. **95**, 60–70.

Tonetta, S.A. & DiZerega, G.S. (1989). lntragonadal regulation of follicular maturation. In *Paracrine Modulation of Folliculogenesis. Endocr. Rev*. **10**, 205–29.

Totey, S.M., Pawshe, C.H. & Appa Rao, K.B.C. (1996). *In vitro* maturation of buffalo oocytes: role of insulin and its interaction with gonadotrophins. *J. Reprod. Fertil*. **50**, 113–19.

Valdez Toledo, C.L. & Pisanó, A. (1980). Fases ovogenéticas en *Bufo arenarum*. *Reproducción* **4**, 315–30.

Wasserman, W.J., Penna, M.J. & Houle, J.G. (1986). The regulation of *Xenopus laevis* oocyte maturation. In *Gametogenesis and the Early Embryo* (ed. A.R. Liss & J.G. Gall), pp. 111–30. New York: Plenum Press.

Wasserman, W.J., Freedman, A. & Penna, M.J. (1988). S_6 phosphorylation is regulated at multiple levels in *Xenopus laevis* oocytes. *J. Exp. Zool*. **247**, 263–70.

ERRATUM

Zygote, Volume 9, Issue 1, February 2001, pages 9–14

Min-Kang Wang, Da-Yuan Chen, Ji-Long Liu, Guang-Peng Li and Qing-Yuan Sun, *In vitro fertilisation of mouse oocytes reconstructed by transfer of metaphase II chromosomes results in live births* contained a spelling error within the third named author.

The authors and publisher wish to apologise to our readers for this mistake.