Turkish Journal of Biology

Volume 24 | Number 4 Article 5

1-1-2000

Effects of pH on the Genesis of Membrane PotentialChanges at Fertilization in the Egg of the Frog Ranacameranoi

ŞEREF ERDOĞAN

GÜLAY LOĞOĞLU

KEREM TUNCAY ÖZGÜNEN

TUNCAY ÖZGÜNEN

Follow this and additional works at: https://journals.tubitak.gov.tr/biology

 Part of the Biology Commons

Recommended Citation

ERDOĞAN, ŞEREF; LOĞOĞLU, GÜLAY; ÖZGÜNEN, KEREM TUNCAY; and ÖZGÜNEN, TUNCAY (2000) "Effects of pH on the Genesis of Membrane PotentialChanges at Fertilization in the Egg of the Frog Ranacameranoi," *Turkish Journal of Biology*: Vol. 24: No. 4, Article 5. Available at: https://journals.tubitak.gov.tr/biology/vol24/iss4/5

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Biology by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

Turk J Biol
24 (2000) 725–736
© TÜBİTAK

Effects of pH on the Genesis of Membrane Potential Changes at Fertilization in the Egg of the Frog *Rana cameranoi*[*]

Şeref ERDOĞAN, Gülay LOĞOĞLU, Kerem T. ÖZGÜNEN, T. ÖZGÜNEN
Çukurova University Faculty of Medicine Department of Physiology 01330 Balcalı, Adana-TURKEY

Received: 03.09.1999

Abstract: The apparent change in the egg membrane potential at fertilization gives rise to fertilization potential (FP). FP is the initial, transient electrical block to polyspermy as shown in most species especially those exhibiting external fertilization, and it protects the egg from a second sperm entry until the permanent, mechanical block is set up. Polyspermy is lethal in most species, and for a successful fertilization resting membrane potential (RMP) and FP of the egg have to be held in optimum ranges. Shifts in environmental pH may interfere with a successful fertilization by affecting these bioelectrical potentials. In the present study, we investigated how pH alterations affected the RMP and FP parameters of the egg in the frog, *Rana cameranoi*. Egg membrane potentials were recorded by the conventional microelectrode technique. RMP and FP parameters were evaluated. Ten percent Ringer solution (pH=7.80) was used for the control group. Acidic (Acd) and alkaline (Alk) experimental groups were kept in the Ringer solutions at pH 6.50 and 9.00, respectively.

RMP was found to be significantly more negative in the Acd group ($p<0.05$), but the difference was not significant in the Alk group when compared with the control. The peak FP value was significantly more positive in the Alk group, and less positive in the Acd group. FP duration was significantly prolonged in the Alk group, and shortened in the Acd group, but the latter change was not significant. Fertilized egg membrane potential in the Acd group was significantly more negative when compared with the Alk group, but did not differ significantly when compared with the control.

Overall evaluation of these results led to the conclusion that moderate shifts in pH affected FP parameters significantly, but did not prevent the genesis of the effective electrical block. However, greater changes in pH may prevent the electrical block, leading to increased polyspermy risk. Alterations in environmental pH, which may interfere with successful fertilization in externally fertilizing species such as frogs, may be due to industrial pollution and consequently be hazardous for the ecologic equilibrium.

Key Words: Resting membrane potential, fertilization potential, pH, frog egg.

Rana cameranoi Türü Kurbağa Yumurtasında pH Değişikliklerinin Fertilizasyonda Gerçekleşen Membran Potansiyeli Değişimine Olan Etkileri

Özet: Fertilizasyonla yumurta zarında belirgin bir potansiyel değişimi (*Fertilizasyon Potansiyeli, FP*) meydana gelmektedir. FP, özellikle dış döllenme gösteren canlıların bir çoğunda gösterildiği gibi, polispermiye karşı ilk sırada oluşturulan geçici elektriksel blokdur ve mekanik engel oluşturulana kadar

* This study was supported by Çukurova University Research Fund (TF 98-35).

yumurtayı ikinci bir spermin girişine karşı korur. Polispermi bir çok türde ölümcül olduğu için fertilizasyonun başarılı olabilmesi yumurtanın dinlenim zar potansiyeli (RMP) ve FP'nin optimum değerlerde tutulmasına bağlıdır. Özellikle kurbağa gibi canlılarda ortam pH'sında gözlenebilecek kaymalar biyoelektrik potansiyelleri etkileyerek başarılı bir fertilizasyon gerçekleşmesini etkileyebilmektedir. Bu noktadan hareketle kurbağa yumurtası RMP ve FP parametrelerine pH'daki değişimlerin etkilerinin incelenmesi amaçlandı. Çukurova Bölgesinde yaygın olarak bulunan "*Rana cameranoi*" türü kurbağa yumurtalarının membran potansiyelleri mikroelektrod tekniği kullanılarak incelendi. Fertilizasyondan önceki RMP ile FP parametreleri değerlendirildi. Kontrol olarak %10 Ringer solüsyonu (pH=7.80) kullanılırken, bu solüsyonun pH'sı 9.00 (Alk grubu) ve 6.50'ye (Asit grubu) ayarlanarak sırasıyla alkali ve asit deney grupları oluşturuldu.

Verilerin değerlendirilmesi sonucu RMP, Asit grubunda anlamlı olarak daha hiperpolarize bulunmuş iken ($p<0.05$) Alk grubundaki farklılık anlamlı değildi ($p>0.05$). Pik FP, Alk grubunda anlamlı olarak daha pozitif değerlere ulaşırken Asit grubunda anlamlı olarak daha az pozitif değerlerde kayıt edildi. FP süresi, Alk grubunda anlamlı olarak daha uzun iken Asit grubunda süre kısaldı ancak bu kısalma istatistiksel olarak anlamlı değildi. Fertilize yumurtanın zar potansiyeli Asit grubunda Alk grubuna göre anlamlı olarak daha hiperpolarize iken kontrole göre anlamlı farklılık göstermedi.

Bu sonuçlar çerçevesinde orta düzeyde pH kaymaları FP parametrelerine anlamlı etkilerde bulunabilmesine karşın etkin elektriksel bloğun oluşumunu önlememiştir. Ancak daha büyük pH değişimleri olması durumunda etkin elektriksel bloğun oluşması engellenerek polispermi riski artabilir. pH'daki bu değişimler endüstriyel atıkların çevrede birikmesi sonucu da meydana gelebilir. Böyle bir durumda kurbağa gibi dış döllenme gösteren canlılarda başarılı fertilizasyonu etkileyebilecek düzeye ulaşılırsa ekolojik dengede ciddi bozulmalar meydana gelmesi söz konusu olabilir.

Anahtar Sözcükler: Dinlenim zar potansiyeli, fertilizasyon potansiyeli, pH, kurbağa yumurtası.

Introduction

Fertilization activates some metabolic and ionic processes in the egg, which result in embryonic development. When activated by a sperm, the egg membrane potential changes suddenly within seconds, and fertilization potential (FP) develops (1,2). FP was demonstrated to be a transient electrical block functioning against polyspermy especially in species reproducing externally (1-5), and it blocks second sperm entry until the genesis of the mechanical barrier (cortical reaction) (1,2,6). FP is functional for nearly 10 min in the frog egg (3,7,8) and this duration may be as long as 90 min in the nemertean (6). Shortening of FP duration increases the incidence of the lethal process, polyspermy (6). In addition, apparent potential change in the egg membrane triggered by means other than sperm makes the egg unable to be fertilized by the sperm (1,2,9-12). For this reason, egg resting membrane potential (RMP) has to be held at optimal levels, and FP has to reach a peak value that lasts until the mechanical block is established; both of these processes possess vital importance for a successful fertilization (1,2).

The first change in the egg when it is activated (by a sperm or artificially) is the increase in ooplasmic Ca^{2+}, and it has been demonstrated in all species that were investigated (2,13-24). Increased ooplasmic Ca^{2+} leads to genesis of FP in the frog, sea urchin, fucoid alga (2,11,12,25,26) and to alkaline shift of ooplasmic pH (pH_i) in the frog, sea urchin and hamster (14-18,27-29). Since ooplasmic alkalinization is important for the activation of egg , pH_i has to be held at low levels to prevent activation in externally reproducing species, because their eggs

are directly exposed to alterations in the environmental pH (13,18,30). It is suggested that increased pH_i alone is not sufficient for activation, but it may have a permissive role for the activation of calcium-dependent events (31). Furthermore, it has been demonstrated that pHi increment may lead to increased ooplasmic Ca^{2+} in the Xenopus and sea urchin (27,32,33). Increased pH leads to activation-induced processes in the egg, except cortical granule exocytosis (27).

Frog eggs are directly affected by environmental conditions. Especially alkaline pH changes disturb ooplasmic ionic equilibrium (14,25,27,32-34), and consequently affect activation of the unfertilized egg by the sperm. In the frog, sea urchin and urechis membrane depolarization of the unfertilized egg prevents incorporation of sperm with the egg (1,2,9-12). Therefore, the effect of increasing environmental pollution on the pH environment and thus bioelectrical potentials of the egg makes it more important to demonstrate the influence of this situation on the fertilization processes of externally reproducing species.

In the present study, our aim was to determine how external pH changes, which alter ooplasmic pH and thus influence ionic and metabolic events in the egg produced by fertilization, affect bioelectrical potentials of the *Rana cameranoi* frog egg.

Materials and Methods

Selected frogs of the *Rana cameranoi* species were kept until use at +4 °C , in plastic boxes, which were filled with small amounts of stock solution (35).

Obtaining eggs and spermatozoa; insemination procedure

For induction of ovulation in the sexually mature females, the pituitary glands which were removed from female frogs were homogenated and injected intraperitoneally, and progesteron (Sigma, P-0130) was injected into the thigh muscle (36). The amounts of injected pituitary glands and progesteron were adjusted according to the season (37). The injected frogs were kept at 18 °C for 36 h, or at 25 °C for 24-36 h, and eggs were then obtained by squeezing the cloaca. Mature eggs were selected according to the criteria cited by Rugh (38).

Sperm suspensions were obtained by macerating frog testes in 10% Ringer, 2-5 h after a male was injected with 300 IU human chorionic gonadotropin (hCG, Sigma CG-2) intraperitoneally. Sperms were examined for motility and morphology under a microscope (Nikon) at 400X magnification.

Insemination procedure was carried out as described previously (3). Normal fertilization was scored by a shift in membrane potential towards positive values (within 5 min), rotation (at about 30 min), normal first cleavage (at about 2.5 h), and neural fold stage (7). Eggs that showed membrane depolarization and rotation but did not cleave normally or did not develop until the neural fold stage, were accepted to be activated. Experiments were performed at 21-25 °C.

Control and experimental groups

Control records (n=12) were carried out in standard 10% Ringer solution which contained (in mM): NaCl, 11.1; KCl, 0.19; $CaCl_2$, 0.11; $MgSO_4$, 0.08; NaOH, 0.4; HEPES, 0.25; pH 7.80 (7). 2-(N-cyclohexylamino)ethanesulphonic acid (CHES, Sigma C-2885) was added to this solution to adjust its pH to 9.00 (Alk group, n=9), and the pH of Acd group (n=8) was adjusted to 6.50 by adding 2-(N-morpholino)ethanesulphonic acid (MES, Sigma M-8250). These pH values are consistent with those of similar studies in the literature (pH values 9.0-9.50 and 6.0-6.50) (27,32,33). Records obtained in these solutions served as experimental groups. pH values of the control and experimental groups were checked just before and after the experiments. Fertilization, first cleavage and neural fold development were normal in all these solutions.

Electrophysiological measurements

Intracellular records were obtained by performing the conventional microelectrode technique. The electrode was inserted into the egg by transiently increasing the negative capacitance of the preamplifier (Nihon Kohden MEZ-7200) to produce an oscillating current. Membrane potentials were monitored on a storage oscilloscope (Nihon Kohden VC-10), and recorded on a chart recorder (Palmer Bioscience). The following parameters were investigated from the electrical recordings: resting membrane potential of the unfertilized mature egg (RMP); fertilization time (F_t); peak fertilization potential (FP_p); duration of the fertilization potential (FP_d); membrane potential of the fertilized egg (MP_f). Evaluated parameters of activation potential (AP_p, AP_d, and MP_a) recorded in the Alk group were analogous to those of FP.

Statistical analysis

All averages were expressed as the mean ± SEM. Significance of differences between means was determined using the one way Anova test. The difference between RMP and MP_f values in the same group was evaluated by the paired t-test. The criterion for significance was $p<0.05$.

Results

A sudden, rapid (within seconds), and depolarizing membrane potential change was recorded in the eggs of *Rana cameranoi* frogs at fertilization. This potential change (Fertilization Potential, FP) lasted for 17.0±1.36 min. At the end of this, membrane potential of fertilized egg recovered to a stabilized level (Fig 1A, 2). The pH of the extracellular solution affected RMP and FP parameters significantly. Representative recordings from experimental groups are shown in Fig. 1. Illustration of the parameters drawn by using values obtained from control group, is shown in Fig. 2.

Resting membrane potential (RMP)

RMP of eggs were investigated until they were stabilized. In the control and Alk groups, RMP values were -31.67 ± 0.94 mV (n=12) and -35.0 ± 0.47 mV (n=9), respectively; this difference was found to be nonsignificant ($p>0.05$, Table 1). The RMP value in the Acd group

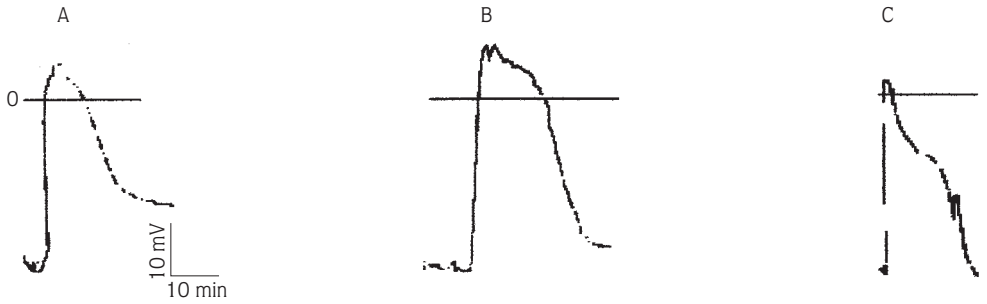

Figure 1. Illustration of records taken from control (A), Alk (B), and Acd (C) groups.

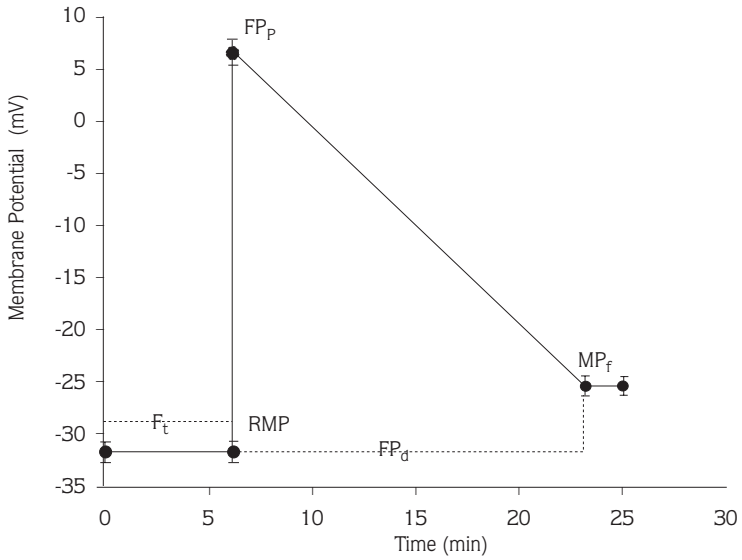

Figure 2. Graphical illustration of evaluated parameters drawn by using mean values determined in the control group.

was determined to be -43.88 ± 1.27 mV (n=8), and the difference was significant ($p<0.05$, Table 1).

While recording RMP traces in the alkaline medium, some of the eggs developed activation soon after the microelectrode was inserted. Potential changes recorded in these eggs (Activation Potential, AP) showed great similarity with FP parameters obtained in the Alk group (Table 2). Such an event was not observed in the Acd group.

Fertilization potential parameters

Fertilization time (F_t): In the control group, F_t was found to be 6.20 ± 0.37 min. This value in the experimental groups did not differ significantly (Table 1).

Peak fertilization potential (FP_p): FP_p was found to be 6.42 ± 1.23 mV in the control group. FP_p was significantly more positive in the Alk group and less positive in the Acd group (12.22 ± 1.88 mV and 1.0 ± 1.27 mV, respectively; $p<0.05$) (Table 1, Fig. 3).

Duration of fertilization potential (FP_d): FP_d was found to be 17.00 ± 01.36 min in the control group. It was significantly prolonged in the Alk group (25.08 ± 02.13 min, $p<0.05$; Table 1). In the Acd group, FPd was shortened, but this difference was not significant when compared with the control (13.52 ± 0.49 min, $p>0.05$; Table 1, Fig. 3).

Membrane potential of fertilized egg (MP_f): In the control group, it was -25.42 ± 0.86 mV, a more positive value than the RMP. In all groups, differences between RMP and MP_f values were found to be significant. In the Acd and Alk groups, the mean MP_f values were -31.63 ± 2.9 mV and -23.56 ± 1.92 mV, respectively; the difference was significant ($p<0.05$, Table 1, Fig. 3). Neither value differed significantly when compared with the control.

RMP and all FP parameters, except F_t, differed significantly between the Alk and Acd groups (Table 1). All parameters belonging to eggs that showed activation during recording in the alkaline medium did not differ from FP parameters of the Alk group (Table 2).

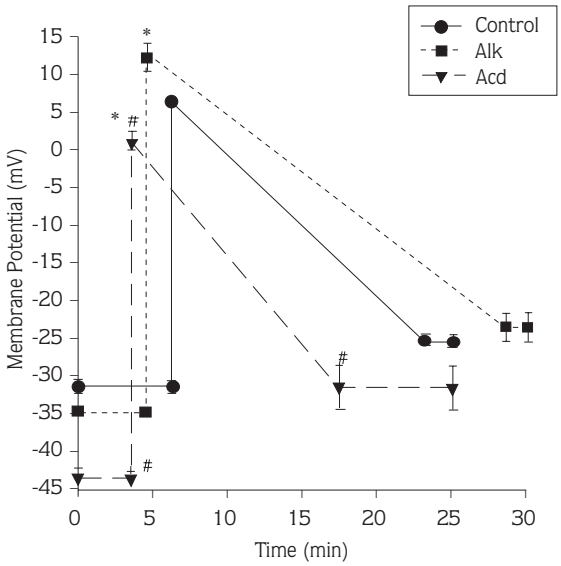

Figure 3. Comparison of values (Mean ± SEM) determined in the control, Alk and Acd groups (*; significant compared with control, #; significant compared with Alk group; $p<0.05$).

Table 1. RMP and FP parameters in the control and experimental groups (Mean±SEM).

Groups	RMP (mV)	F_t (min)	FP_p (mV)	FP_d (min)	MP_f (mV)
Control (n=12)	-31.67±0.94	06.20±0.37	6.42±1.23	17.00±1.36	-25.42±0.86$^{\Phi}$
Alkali (n=9)	-35.0±0.47	04.55±0.41	12.22 ± 1.86*	25.08 ± 2.13*	-23.56±1.92$^{\Phi}$
Acid (n=8)	-43.88±1.27*$^{\#}$	03.53±0.30	1.0±1.27*$^{\#}$	13.52±0.49$^{\#}$	-31.63±2.9$^{\#\Phi}$

*; significant compared with control ($p<0.05$).

#; significant compared with Alk group ($p<0.05$).

Ø; significant compared with RMP of the same group ($p<0.05$).

Table 2. Fertilization- and activation- induced membrane potential changes recorded in alkali (pH=9.00) medium (Mean±SEM).

	RMP (mV)	FP_p or AP_p (mV)	FP_d or AP_d (min)	MP_f or MP_a (mV)
Fertilization (n=9)	-35.0±0.47	12.22±1.86	25.08±2.13	-23.56±1.92
Activation (n=8)	-34.38±3.35	13.63±1.81	22.05±2.26	-25.75±1.3

Discussion

The findings of the present study prove that shifts in pH significantly affect bioelectrical potentials of the frog egg which are important for its fertilization; however these influences do not prevent the effective block against polyspermy.

Resting membrane potential (RMP)

In the present study, RMP did not differ significantly except in the Acd group (Table 1). In a study with Xenopus eggs recorded in alkaline medium, when pH was adjusted to 9.00 by adding NH_4^+, the membrane depolarized to 7 mV after about 30 min, leading to inhibition of sperm-egg incorporation as long as this strong alkali exposure was continued (33). In another study with hamster eggs (32), medium at pH 9.50 resulted in approximately 40 mV hyperpolarization beginning within 1 min; this hyperpolarization was due to increased K^+ conductance, and lasted as long as pH was maintained at this alkali value. In contrast to these changes observed in other species, our groups studied at pH values of 9.00 and 6.50 did not exhibit depolarization to the extent to prevent incorporation of sperm with the egg. On the other hand, membrane hyperpolarized significantly when the pH of the solution was adjusted to 6.50. However, this hyperpolarization was not sufficient to prevent fertilization of the frog egg. The effect of acidic pH on RMP was also demonstrated in the hamster egg (32), and 10 mV depolarization of

membrane was recorded at pH values of 6.50 and 5.00. Since the RMP of the frog egg is mainly affected by K^+ gradient (1-3,7,8), the membrane hyperpolarization in an acidic environment determined in our study may be due to increased K^+ conductance. The effect of acidity on K^+ channels may be via altering some of their properties such as configuration.

Fertilization time (F_t)

In the present study, the mean F_t value in the control group was 6.42±1.23 min (Table 1, Fig.2). Similar results were found in the literature (3,35,39). F_t values determined in our study did not differ significantly in either of the experimental groups. However, it is known that sperm penetration is facilitated by the alkali pH of the female reproductive tract, and that optimal fertilization rates are achieved in IVF procedures when the pH of media are adjusted to alkaline values (pH=7.0 – 8.50) (32). The lack of pH influence on F_t determined in our study may be due to direct exposure of eggs with the sperms (artificial insemination), leading to sperm attachment to the vitelline membrane directly.

Peak fertilization potential (FP_p)

FP_p value of the control group determined in the present study was similar to those obtained from *Rana cameranoi, Rana pipiens* and *Xenopus laevis* eggs (3,35,39,40). FP_p recorded in the alkali medium was significantly more positive (Table 1, Figure 3), which indicates that the effect of the ion responsible for depolarization is potentiated in an alkali environment. Fertilization triggers Cl^- conductance increment that leads to depolarization in the frog egg (1-3,7,8,19,41). Ooplasmic pH increment has been shown to increase intracellular Ca^{2+} by decreasing Ca^{2+} pump activity in the oolemma (32); so it may be concluded that increased ooplasmic Ca^{2+} triggered by fertilization, as well as by alkali pH makes the Ca^{2+}-induced increase of Cl^- conductance more potentiated. Equilibrium potential of Cl^- in the frog egg is +18 mV (7), and potentiated Cl^- effect may lead to more positive FP_p values (3). Unfertilized eggs that we recorded in alkali medium were readily activated, and this may indicate that ooplasmic Ca^{2+} increase caused together by alkali pH and by diffusion of Ca^{2+} from the cell exterior to interior (spontaneous activation due to mechanical irritation)(14) triggers an increase in Cl^- conductance. This conclusion explains more positive FP_p values that we recorded in the alkali medium.

The less positive FP_p value determined in the Acd group may be due to increased Ca^{2+} pump activity in acid pH, leading to decreased ooplasmic Ca^{2+}, which is in contrast to the effect of an alkali environment. If the effect of pH on the level of ionized calcium is kept in mind (42), it can be concluded that decreased ionized form in alkali pH may act to depress Ca^{2+} pump activity to prevent further removal of this vital ion from the cell interior, whereas increased ionized form in acid pH may act to increase Ca^{2+} pump activity. As a result, decreased ooplasmic Ca^{2+} at low pH may decrease Cl^- conductance, which in turn causes repolarizing K^+ conductance to become more evident, leading to a significantly decreased FP_p value. However, decreased FP_p values recorded in solution at pH 6.50 prevented genesis of the effective block in neither of the eggs (the least negative FP_p value recorded in this group was –5 mV; in the frog, membrane potential values leading to electrical block have been demonstrated to be more positive than –10 mV)(10).

Duration of fertilization potential (FP_d)

The mean FP_d value in the control group was found to be 17.00±1.36 min, which is in accordance with other findings in the literature (3,35,39,40). FP_d was significantly prolonged in the Alk group; the shorter duration determined in the Acd group was not significant (Table 1, Fig. 3). The significant prolongation in the Alk group may be explained by more dominant depolarizing force (increased Cl^- conductance) in this group compared with the control, leading to more prolonged repolarization.

Nonsignificantly shortened FP_d in the Acd group partly supports increased Ca^{2+} pump activity in acid pH, the view that has been mentioned above. The rapid removal of ooplasmic Ca^{2+}, which will cause the effect of depolarizing force to decrease, will then result in a shorter time for repolarizing force (increased K^+ conductance) to exhibit its effect. In spite of shortened FP_d, development of the eggs revealed that the effective electrical block was not prevented in the acid environment.

Membrane potential of fertilized egg (MP_f)

Fertilized egg membrane was significantly more depolarized in all groups, when compared with RMP values (Table 1). This finding has been determined in the majority of similar studies (2,3,35,40), and concluded to be due to genesis of leakage currents during recording by microelectrode (43).

MP_f value differed significantly in the Acd group, when compared with the Alk group (Table 1, Fig. 3). This value did not show significant difference when compared with the control (Table 1), but the more negative MP_f value determined in the Acd group may be due to the effects of acidity on K^+ channels, as also concluded to be the reason of hyperpolarization determined in the unfertilized egg membrane at acidic pH.

Conclusion

Overall evaluation of our findings principally reveals that shifts in environmental pH have significant effects on the egg bioelectrical potentials that play an important role during fertilization. However, the same pH shifts do not prevent the effective electrical block. On the other hand, our findings indicate that the greater alterations in pH values may prevent the genesis of a successful fertilization in the species that show external fertilization. Therefore, it has to be kept in mind that environmental pollution increasing in parallel with industrial development may seriously affect the pH of water sources and thus the reproduction of species such as frogs.

References

1. Jaffe, L.A., Electrical regulation of sperm-egg fusion. Ann. Rev. Physiol. 48:191-200, 1986.
2. Erdoğan, Ş., Loğoğlu, G., Özgünen, T., The importance of bioelectrical potentials at fertilization. Ann Med Scien 8:58-62, 1999.

3. Erdoğan, Ş., Loğoğlu, G., Özgünen, T., The ionic basis of membrane potential changes from before fertilization through the first cleavage in the egg of the frog, *Rana cameranoi*. Gen Physiol Biophys 15:371-387, 1996.

4. Kline D., Jaffe, L.A., Kado, R.T., A calcium-activated sodium conductance contributes to the fertilization potential in the egg of the Nemertean Worm *Cerebratulus lacteus*. Dev Biol 117:184-93, 1986.

5. Goudeau, H., Goudeau, M., Long-lasting electrically mediated block, due to the egg membrane hyperpolarization at fertilization, ensures physiological monospermy in eggs of the crab *Maia squinado*. Dev Biol 133:348-360, 1989.

6. Kline, D., Jaffe, L.A., Tucker, R.P., Fertilization potential and polyspermy prevention in the egg of the Nemertean, *Cerebratulus lacteus*. J of Exp Zool, 236:45-52,1985.

7. Jaffe, L.A., Schlichter, L.C., Fertilization-induced ionic conductances in eggs of the frog, *Rana Pipiens*. J of Physiol 358:299-319, 1985.

8. Jaffe, L.A., Kado, R.T., Muncy, L., Propagating potassium and chloride conductances during activation and fertilization of the egg of the frog, *Rana pipiens*. J Physiol 368: 227-242, 1985.

9. Iwao, Y., Jaffe, L.A., Evidence that the voltage-dependent component in the fertilization process is contributed by the sperm. Dev Biol 134:446-451, 1989.

10. Iwao, Y., The membrane potential changes of amphibian eggs during species- and cross-fertilization. Dev Biol 111: 26-34, 1985.

11. Brawley, S.H., A sodium-dependent, fast block to polyspermy occurs in eggs of fucoid algae. Dev Biol 124:390-397, 1987.

12. Brawley, S.H., The fast block against polyspermy in Fucoid algae is an electrical block. Dev Biol 144:94-106, 1991.

13. Ben-Yosef, D., Oron, Y., Shalgi, R., Intracellular pH of rat eggs is not affected by fertilization and the resulting calcium oscillations. Biol of Reprod, 55: 461-468, 1996.

14. Grandin, N., Charbonneau, M., Intracellular pH and the increase in protein synthesis accompanying activation of Xenopus eggs. Biol of the Cell, 67: 321-330, 1989.

15. Grandin, N., Charbonneau, M., The increase in intracellular pH associated with Xenopus egg activation is a Ca^{2+} -dependent wave. J of Cell Scien, 101:55-67, 1992.

16. Shina, Y., Keneda, M., Matsuyama, K., et al., Role of the extracellular Ca^{2+} on the intracellular Ca^{2+} changes in fertilized and activated mouse oocytes. J Reprod Fert, 97:1,1-150, 1993.

17. Kühtreiber, W.M., Gillot, I., Sardet, C., Jaffe, L.F., Net calcium and acid release at fertilization in eggs of sea urchins and ascidians. Cell Calcium, 14: 73-86, 1993.

18. Phillips, K.P., Baltz, J.M., Intracellular pH regulation by HCO_3/Cl^- exchange is activated during early mouse zygote development. Dev Biol, (15) 208:2 392-405, 1999.

19. Machaca, K., Hartzell, H.C., Asymmetrical distribution of Ca-activated Cl channels in Xenopus oocytes. Biophys J, 74:(3) 1286-95, 1998.

20. Suzuki, K., Tanaka, Y., Nakajima, Y., et al., Spatiotemporal relationships among early events of fertilization in sea urchin eggs revealed by multiview microscopy. Biophys J., 68:3 739-48, 1995.

21. Gillot, I., Payan, P., Girard, J.P., Sardet, C., Calcium in sea urchin egg during fertilization. Int J Dev Biol 34:1,117-125, 1990.

22. Miyazaki, S., Repetitive calcium transients in hamster oocytes. Cell Calcium, 12:205-216, 1991.

23. Lawrence, Y., Whitaker, M., Swann, K., Sperm-egg fusion is the prelude to the initial Ca^{+2} increase at fertilization in the mouse. Development 124:233-241, 1997.

24. Sun, F.Z., Bradshaw, J.P., Galli, C., Changes in intracellular calcium concentration in bovine oocytes following penetration by spermatozoa. J Reprod Fertil, 101:3,713-9, 1994.

25. Dufresne, L., Swezey, R.R., Epel, D., Kinetics of actin assembly attending fertilization of artificial activation of sea urchin eggs. Exp Cell Res, 172:1 32-42, 1987.

26. Whitaker, M.J., Steinhardt, R.A., Evidence in support of the hypothesis of an electrically mediated fast block to polyspermy in sea urchin eggs. Dev Biol 95:244-248,1983.

27. Charbonneau, M., Webb, D.J., Weak bases partially activate Xenopus eggs and permit changes in membrane conductance whilst inhibiting cortical granule exocytosis. J Cell Scien, 87:205-220, 1987.

28. Webb, D.J., Nuccitelli, R., Direct measurement of intracellular pH changes in Xenopus eggs at fertilization and cleavage. J Cell Biol, 91:2 562-7, 1981.

29. Gould, M.C., Stephano, J.L., Nuclear and cytoplasmic pH increase at fertilization in *Urechis caupo*. Dev Biol, 159:2 608-17, 1993.

30. Dale, B., Menezo, Y., Cohen, J., DiMatteo, L., Wilding, M., Intracellular pH regulation in the human oocyte. Hum Reprod, 13:4 964-70, 1998.

31. Dube, F., The relationships between early ionic events, the pattern of protein synthesis, and oocyte activation in the surf calm, *Spisula solidissima*. Dev Biol, 126:233-241, 1988.

32. Georgiou, P., House, C.R., McNiven, A.I., Yoshida, S., On the mechanism of a pH-induced rise in membrane potassium conductance in hamster eggs. J of Physiol, 402:121-138, 1988.

33. Charbonneau, M., Webb, D.J., Multiple activation currents can be evoked in *Xenopus laevis* eggs when cortical granule exocytosis is inhibited by weak bases. Pflügers Arch, 407: 370-376, 1986.

34. Ciapa, B., Allemand, D., Payan, P., Girard, J.P., Sodium-potassium exchange in sea urchin egg. II. Ionic events stimulating the Na^{+}-K^{+} pump activity at fertilization. J Cell Physiol, 121:1 243-50, 1984.

35. Webb, D.J., Nucitelli, R., A comparative study of the membrane from before fertilization through cleavage in two frogs, *Rana Pipiens* and *Xenopus Laevis*. Comp Biochem Physiol 82A;No.1:35-42, 1985.

36. Perkins, K.W., Whitten, R.H., Reptiles and Amphibians: Care and Culture. USA, 1981, Carolina Biological Supply Company, 9-11.

37. Rugh, R.: Experimental embryology techniques and procedures. USA, 1962 (Third Edition), Burgess Publishing Company, 93.

38. Rugh, R., The frog; Its reproduction and development. USA, 1951, McGraw-Hill Book Company, 67-71.

39. Webb, D.J., Nucitelli, R., Fertilization potential and electrical properties of the *Xenopus laevis* egg. Dev Biol 107: 395-406, 1985.

40. Erdoğan, Ş., Loğoğlu, G., A comparative study of fertilization- and activation-induced membrane potential changes in the frog egg, *Rana cameranoi*. The periodical of Cukurova University Faculty of Medicine (Turkish) 2: 68-73, 1996.

41. Kline, D., Calcium-dependent events at fertilization of the frog egg: Injection of a calcium buffer blocks ion channel opening, exocytosis, and formation of pronuclei. Dev Biol 126: 2, 346-61, 1988.

42. Ganong, W.F., Review of medical physiology. USA, 1997 (18th Edition), Appleton & Lange Company, 359.

43. Peres, A., Bernardini, G., Negrini, C., Membrane potential measurements of unfertilized and fertilized Xenopus eggs are affected by damage caused by the electrode. Exp Cell Research, 162: 159-168, 1986.