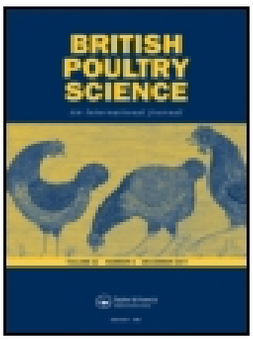

British Poultry Science

ISSN: 0007-1668 (Print) 1466-1799 (Online) Journal homepage: http://www.tandfonline.com/loi/cbps20

Heart rate in developing ostrich embryos

H. TAZAWA , A. AR , J.T. PEARSON , K. MORIYA & E. GEFEN

To cite this article: H. TAZAWA , A. AR , J.T. PEARSON , K. MORIYA & E. GEFEN (1998) Heart rate in developing ostrich embryos, British Poultry Science, 39:1, 161-166, DOI: 10.1080/00071669889565

To link to this article: http://dx.doi.org/10.1080/00071669889565

British Poultry Science (1998) **39:** 161–166

Heart rate in developing ostrich embryos

H. TAZAWA, A. AR,[1] J. T. PEARSON, K. MORIYA AND E. GEFEN[1]

Department of Electrical and Electronic Engineering, Muroran Institute of Technology, Muroran, Japan and Department of Zoology, Tel Aviv University, Israel

Abstract 1. A non-invasive condenser microphone was used to detect cardiogenic, acoustic pressure changes (acoustocardiogram, ACG) over the eggshell in order to determine embryonic heart rate (HR) of ostriches in a commercial hatchery.
2. HR measured for 36 eggs at 36·3°C was maintained at about 185 bpm during the middle stage of development (days 19 to 23) and then decreased with embryonic development.
3. The daily changes in HR were not related to egg mass, but HR of high water vapour conductance ($GspH_2O$) eggs was found to decrease less during the last stages of incubation relative to low and medium $GspH_2O$ groups.
4. The averaged HR at 80% of incubation period was close to the value predicted from the allometric equation determined previously for embryos of domesticated precocial birds.

INTRODUCTION

The ostrich (*Struthio camelus*) has become a commercially farmed animal in several countries (Deeming *et al.*, 1993) including Israel where such farming is well established. In order to improve hatchability, research and development take place in the ostrich hatcheries. Water vapour conductance of the eggshell (GH_2O) together with initial egg mass is determined in individual eggs, and eggs with relatively different GH_2O are incubated in separate incubators provided with different humidities in an attempt to maintain adequate water loss.

We have developed various non-invasive methods and techniques to determine the embryonic heart rate (HR) of birds, and have been able to measure developmental patterns of HR (daily changes in mean HR during incubation) in several species of altricial and precocial birds and also in semi-precocial seabirds (Tazawa *et al.*, 1990; Tazawa *et al.*, 1991; Burggren *et al.*, 1994; Tazawa *et al.*, 1994 Tazawa and Whittow, 1994). We were interested in measuring the embryonic HR during normal incubation of the largest living bird in terms of its developmental pattern and allometric relationship with egg mass. The developmental patterns of prenatal HR of domesticated precocial birds show a trend to decrease towards pipping time (Laughlin *et al.*, 1976; Tazawa *et al.*, 1991). Thus it was predicted that the daily HR of ostrich embryos would also decrease during the last stages of incubation and mean HR would be around 160 beats/min. The present study on non-invasive determination of embryonic HR of the ostrich in relation to eggshell GH_2O and egg mass was undertaken in an ostrich hatchery in Israel.

MATERIALS AND METHODS

Incubated eggs

Experiments were carried out at a hatchery of Zemach Ostriches, Israel, for a limited period of 5 consecutive days from March 3, 1996, when routine commercial incubation was in operation. Eggs collected from farms were kept in a storing room until set for incubation once a week and measured for mass and GH_2O. The first day of starting incubation was counted as day 0 and GH_2O was measured as described below. Each egg was marked and weighed just before being set. Eggs were incubated for 10 to 15 d in an incubator of a given relative humidity (*c.* 30%) and temperature (*c.* 36·3°C). Incubation temperature and humidity were recorded hourly and averaged for the entire period. At the end of this period, eggs were weighed again and mass specific $GH_2O(GspH_2O)$ values were calculated from egg mass, mass loss, internal egg humidity (calculated from water vapour saturation pressure at the averaged incubator temperature), averaged incubator water vapour pressure (calculated from the averaged incubator temperature and relative humidity) and corrected for the averaged barometric pressure and standard temperature (Meir *et al.*, 1984; Meir and Ar, 1987; *et al.*, 1996). Using these $GspH_2O$ values eggs were grouped into low, medium and high $GspH_2O$ eggs and were directed into 1 of 3 incubators with humidity set at an appropriate level to achieve an average of 13% water loss, until transferred to the hatcher on d 38 of incubation.

For our experiments, we selected 12 eggs from each of the egg groups incubated already for 19 d

Correspondence to: Hiroshi Tazawa, Department of Electrical and Electronic Engineering, Muroran Institute of Technology, Muroran 050, Japan.

Accepted for publication 30th June 1997

0007-1668/98/010161-06 $7.00

(group I), 26 d (group II) and 33 d (group III), respectively to make 3 age groups. Samples of eggs were selected to contain eggs with small and large masses, and small, medium and large GH_2O values. Thus, embryonic HR was measured for eggs of different ages during the same 5-d period, under the same incubator conditions. The average incubation duration in this hatchery was 41 d.

Group I: HR was measured every day from 19 to 23 d of incubation; equivalent to 46% to 56% of the incubation duration. The mean mass of the 12 eggs was 1320 ± 189 (SD) g, ranging from 1037 to 1578 g. The mean value of $GspH_2O$ of the 12 eggs was $103{\cdot}8 \pm 19{\cdot}7$ mg/(d.Torr.kg). Mean $GspH_2O$ of the 3 subgroups (small, medium and large) was $80{\cdot}8 \pm 2{\cdot}2$ ($n = 4$; numbered from I–1 to I–4 in increasing order of egg mass), $102{\cdot}0 \pm 2{\cdot}6$ ($n = 4$; numbered from I–5 to I–8) and $128{\cdot}7 \pm 1{\cdot}0$ ($n = 4$; numbered from I–9 to I–12), respectively.

Group II: HR was measured every day from d 26 to 30 d of incubation; equivalent to 63% to 73% of the incubation duration. Mean egg was 1334 ± 182 g ($n = 12$), ranging from 1107 g to 1588 g. The mean $GspH_2O$ was $110{\cdot}1 \pm 29{\cdot}6$ mg/(d.Torr.kg) for 12 eggs and $75{\cdot}9 \pm 2{\cdot}3$ ($n = 4$; from II–1 to II–4), $106{\cdot}6 \pm 3{\cdot}7$ ($n = 4$; from II–5 to II–8) and $147{\cdot}7 \pm 5{\cdot}1$ ($n = 4$; from II–9 to II–12) for the 3 subgroups, respectively.

Group III: HR was determined every day from 33 to 37 d of incubation; equivalent to 80% to 90% of the incubation duration. Mean egg mass was 1331 ± 103 g ($n = 12$), ranging from 1128 g to 1499 g. Mean $GspH_2O$ was $106{\cdot}0 \pm 20{\cdot}5$ mg/(d.Torr.kg) for 12 eggs and $82{\cdot}8 \pm 7{\cdot}4$ ($n = 4$; from III–1 to III–4), $103{\cdot}8 \pm 2{\cdot}9$ ($n = 4$; from III–5 to III –8) and $131{\cdot}6 \pm 1{\cdot}3$ ($n = 4$; from III–9 to III–12) for the 3 subgroups, respectively.

Measurement procedures

Two eggs were transferred from the commercial incubator to an experimental chamber (taking less than one min). The chamber comprised a wooden thermostatted box 60×60 cm wide and 30 cm high, covered with a removable transparent plastic dome. Two round windows, 10 cm in diameter, were cut through the front panel of the chamber and covered with rubber flaps to enable manipulation of the eggs and the ACG pickup (a condenser microphone). The temperature in the chamber was kept at $36{\cdot}3\,^{\circ}C$ by a thermostatted electric heater and fan. The measurement for the first egg began after about 10 min and took about 10 min for each egg. After 2 eggs were measured, they were returned to the commercial incubator and another 2 eggs introduced into the experimental chamber. Thirty six eggs were measured each day.

Acoustocardiogram

From the various available methods for non-invasive determination of embryonic HR, acoustocardiography was used because it was relatively free from external noises (vibrations) compared with other measuring systems (Rahn *et al.*, 1990; Wang *et al.*, 1990; Haque *et al.*, 1994; Akiyama *et al.*, 1997).

A conventional condenser microphone was used to detect cardiogenic acoustic pressure changes occurring outside the eggshell through the pores (that is, acoustocardiogram, ACG). The condenser microphone was covered with a cylindrical metal frame with a 2 mm hole across in the centre of the front surface. The microphone was placed on the eggshell so that the hole fitted over visible pores and was sealed hermetically on to the eggshell with clay. The microphone was biased by a 6-volt battery and was connected to a Grass polygraph amplifier. The output was monitored by an oscilloscope. If no ACG signal was detected, the microphone was repositioned on the eggshell until the required signal was obtained. The amplifier was set at selected amplifications ranging from 0·2 mV/cm to 5 mV/cm so that ACG waves could be observed and recorded as large deflections on the polygraph chart.

Mean heart rate

Once the ACG signals were seen on the oscilloscope, the polygraph was switched to recording position at a paper speed of 5 mm/s. Continuous recordings of no less than 30 s were made every 2 min for at least 10 min. This recording was sectioned every 25 min (5 s section). The number of ACG waves in each 5 s section was counted and the time interval between the 2 peaks of the first and last ACG waves in the section was determined. A value of HR in beats per min (bpm) for the approximate 5 s period (referred to as HR_5) was then calculated. Six to 7 values of HR_5 were determined from a single recording and averaged to give a mean value (referred to as HR_{30}) for an approximate 30 s period. This procedure revealed variations in HR_5 during the 30 s period. From each of the 30 s recordings made every 2 min for each egg, 5 to 6 values of HR_{30} were determined and averaged again to give a mean value of HR for an embryo on a given incubation day (referred to as MHR).

Statistical analysis

Differences in HR between the 3 age groups and the effects of egg mass and $GspH_2O$ on the HR were examined by 3 way ANOVA. A 2 way ANOVA examined the significance of daily changes in HR and the effect of GH_2O on the HR. Pairwise comparisons of group means adjusted by

the Bonferroni Procedure were examined for differences between 3 groups to indicate which groups differed from others at the critical level of 0·016 (0·05 divided by number of groups (3)).

RESULTS

Acoustocardiogram and determination of HR_5, HR_{30} and MHR

Figure 1 shows the ACG recorded from an embryo (II–3) on day 28 of incubation and the mean HR (HR_5) calculated for an approximate period of 5 s. The recording is typical of an ACG signal. In this example, the ACG amplitude increased when the second measurement was made (the first 20 s recorded section of the top tracing). When the third measurement was made after the second break (thick arrow), the amplitude of the ACG wave decreased to less than half the previous value and the HR_5 changed considerably. For example, for the 5 s period prior to the thick arrow, 11 peaks were counted during a measured distance of 25·2 mm for time interval and thus calculated HR_5 to be 131 bpm. Similarly, for the first 5 s period of recording at 09·43, 14 peaks were counted during the time interval of 24·8 mm and thus calculated HR_5 to be 169 bpm.

All HR_5 s calculated as above were averaged to give the mean HR for an approximate 30 s recording period (mean $HR_5 = HR_{30}$). In the above embryo (II–3 on day 28), the $HR_{30} \pm SD$ was 179 ± 1 (n = 7), 122 ± 3 (11), 169 ± 5 (13), 172 ± 5 (6), 173 ± 4 (6) and 176 ± 3 (8) bpm. The second HR_{30} value was low compared with the others, but, the coefficient of variation in HR_{30} (2·5%) was similar. Finally, these HR_{30} values were averaged again to give the mean HR on this incubation day (mean HR_{30} = MHR); the MHR ± SD of the embryo in this example was 165 ± 20 bpm (number of mean $HR_{30} = 6$). Because of the unusually low HR_{30} value in the second determination, the SD was high, and the coefficient of variation of MHR was large (12·1%).

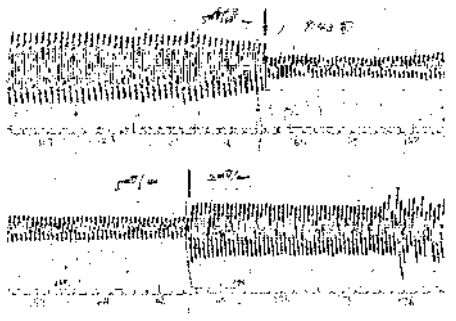

Figure 1. *An example of an acoustocardiogram of a 28-d-old embryo. Measurements at about 30 s intervals were made every 2 min during a 10-min period. The time-marks shown at the bottom of the recording indicate 1–5 intervals and the bottom tracing is continued from the top. The arrow on the top tracing indicates a 2 min break in recording (the upper 1st 20 s recorded section was a part of the 2nd measurement recorded at 09·41 and after the 2 min break marked with the arrow the 3rd measurement was made). These measurements were longer than 30-sec, because the amplitude of ACG increased in the 2nd measurement and the amplification was decreased (5 mV/cm). At the thin arrow at bottom, the amplification was restored to 2 mV/cm to obtain a sharp and large deflection of the ACG wave. The numbers written under the time-mark indicate the HR_5 values.*

Variation in heart rate during development

Such an abrupt change in HR_{30} as shown in Figure 1 did not occur in group I embryos, only featured once in group II, but occurred frequently in many eggs of group III as shown in Figure 2 which presents an example of HR_{30} variation. The variation in MHR increases late in incubation (group III) in all 3 groups of low (panel A), medium (panel B) and high (panel C) $GspH_2O$ eggs. The MHR ± SD of egg II–3, whose ACG is shown in Figure 1, was $182 \pm 0{\cdot}4$ ($n = 5$), 178 ± 2 (5), 165 ± 20 (5), 165 ± 2 (5) and 160 ± 2 (5) bpm for incubation ages of 26, 27, 28, 29 and 30 d, respectively. The variation in HR_{30} was small except for day 28. In another example, MHR ± SD in egg III–4 was 146 ± 2 ($n = 5$), 147 ± 6 (6), 119 ± 19 (5), 119 ± 7 (6) and 108 ± 4 (7) bpm on days 33, 34 35, 36 and 37 of incubation. HR variation in this age group (III) was high compared to the younger groups. Figure 2 also indicates that MHR decreased with embryonic development.

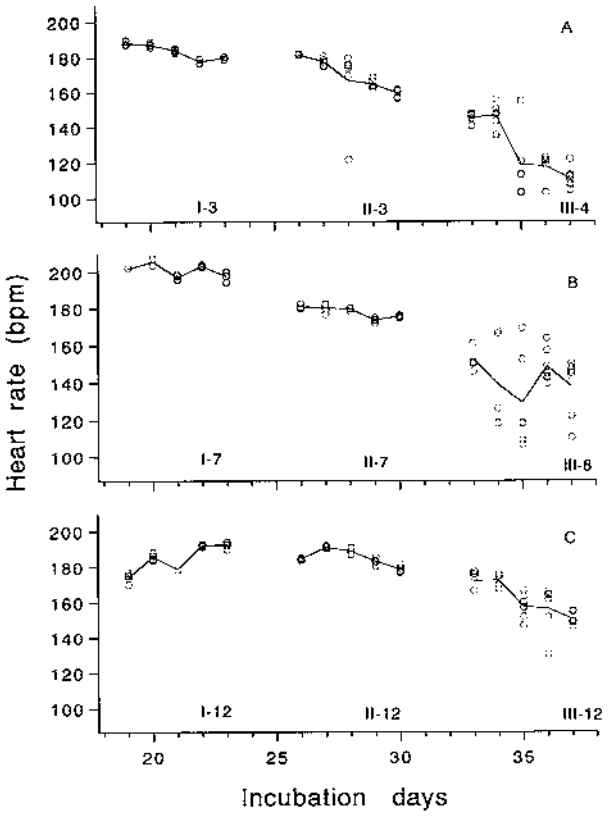

Figure 2. *Variation in HR_{30} (averaged HR_5) of 12 eggs, representing the age groups I, II and III. Each open circle presents the HR_{30}. Solid lines connect the MHR (mean HR_{30}) of individual eggs in each group.*

Developmental patterns of MHR

Figure 3 presents developmental patterns of MHR determined in 36 embryos. The 3 way ANOVA indicated that the MHR was significantly different between groups I, II and III ($F = 205{\cdot}4$, $P < 0{\cdot}0001$); thus indicating that the MHR decreased significantly with age, and between panels A, B and C ($F = 9{\cdot}08$, $P < 0{\cdot}0001$); indicating that the MHR was affected by $GspH_2O$. There was no significant relationship with egg mass ($F = 0{\cdot}19$). The interaction between groups (age) and $GspH_2O$ was significant ($F = 4{\cdot}71$, $P < 0{\cdot}001$). The 2 way ANOVA tested for differences in MHR between individual incubation days, and between $GspH_2O$'s in each group. In group I (19 to 23 d), there were no significant differences in MHR within incubation days, nor between the 3 $GspH_2O$ groups. In group II (26 to 30 d), the differences in MHR between incubation days and between $GspH_2O$ groups were significant ($F = 9{\cdot}29$, $P < 0{\cdot}0001$ and $F = 14{\cdot}21$, $P < 0{\cdot}0001$, respectively), but the interaction was not. In group III (33 to 37 d), the difference in MHR between incubation days was significant ($F = 11{\cdot}79$, $P, < 0{\cdot}0001$) and $GspH_2O$ also affected the MHR ($F = 10{\cdot}60$, $P < 0{\cdot}0001$). The results of the 2 way ANOVA indicated that the MHR decreased significantly from 26 to 30 d and from 33 to 37 d. In addition, $GspH_2O$ significantly affected the MHR in groups II and III. For groups II and III, in order to determine which group of $GspH_2O$ (low, medium or high) differed from the others, pairwise comparisons of the 3 $GspH_2O$ groups were made by the Bonferroni Adjustment. For group II, the pairwise comparison of HR between low and medium $GspH_2O$ groups was not significant, but the MHR of high $GspH_2O$ group was significantly higher than that of medium and low $GspH_2O$ groups. Similarly, in group III eggs, the difference in HR between low and medium $GspH_2O$ groups was not significant, but the high $GspH_2O$ group had a significantly higher MHR than medium and low $GspH_2O$ groups.

The Bonferroni Adjustment was used to examine the differences in developmental pattern of MHR between low, medium and high $GspH_2O$ groups across all age groups (that is, between the 3 developmental patterns in panels A, B and C in Figure 3). The high $GspH_2O$ group had a significantly higher HR than medium and low $GspH_2O$ groups. The differences between the medium and the low $GspH_2O$ groups were also significant.

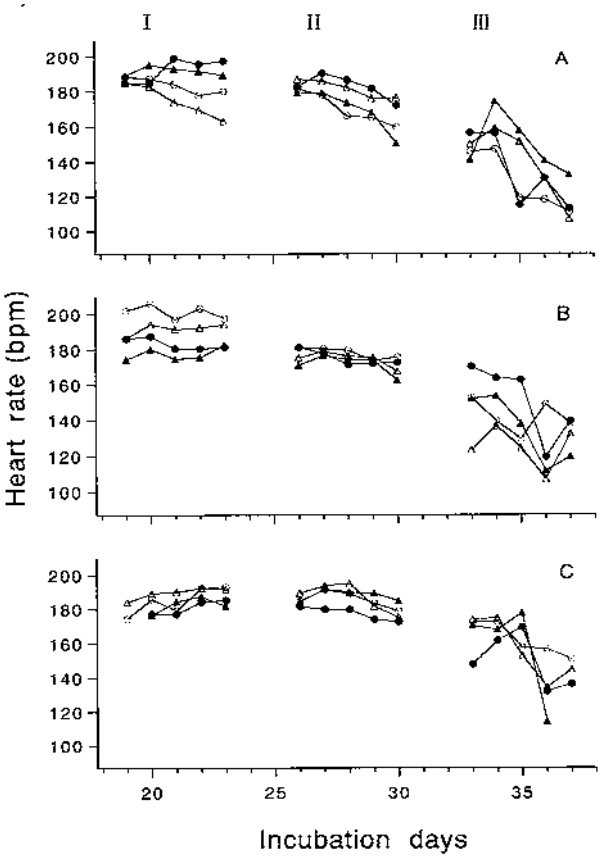

Figure 3. *The developmental patterns of MHR in 3 groups of eggs (columns I, II and III) having low (panel A), medium (panel B) and high (panel C) $GspH_2O$. Individual egg values are connected by solid lines. The 12 eggs presented in Figure 2 are shown by open circles.*

DISCUSSION

Recording of ACG

Although it is recognised that the ACG is cardiogenic and can be detected as a cyclic signal through the eggshell pores, its mechanism of pressure changes is controversial (Rahn *et al.*, 1990; Wang *et al.*, 1990). For appropriate detection, the front surface of the microphone must be sealed hermetically over a selected area of pores. The amplitude of the ACG signal was dependent upon the developmental stages of embryos and varied between individual embryos. In some embryos of group I, it was difficult to detect the ACG during the first days of measurement (19 to 21 d). In 2 eggs, the ACG could not be measured on 19 d of incubation, but was detected later at the amplication of 0·2 mV/cm, depending upon the location of the microphone on the eggshell. The ACG signals strengthened with embryonic development and could be measured at any site on the eggshell at a low amplification in all embryos of age groups II and III.

Although the microphone covered over a small part of the eggshell (*c.* 2 cm^2), we are confident that it did not affect adversely the gas exchange in the whole egg. A previous report on a catheterisation of the allantoic blood vessels of chicken eggs, which impeded gas exchange through an almost identical surface area, showed that the blood gas properties (Po_2, Pco_2 and pH) are not affected by partial impediment to gas exchange (Tazawa *et al.*, 1980; Tazawa, 1981*a*;

1981*b*). In addition, the attachment of the microphone on the shell of chicken eggs did not adversely affect the determination of embryonic HR (Haque *et al.*, 1994). Chicken eggs have a much smaller shell surface area than ostrich eggs, so we are sure that the ACG method does not interfere with normal development of ostrich embryos.

Variation in heart rate

Variation in HR_{30} was greater in group III eggs than in group I and II eggs (Figure 2). This implies that up to about 75% of the incubation period, the embryonic HR remained relatively stable for at least 10 min, but after about 80% of incubation, it varied from min to min. Increasing variation in HR with embryonic development is also found in domesticated birds (Tazawa *et al.*, 1991; Akiyama *et al.*, 1997). Although embryonic activity increases towards the end of incubation, it is not related to HR variability (Akiyama *et al.*, 1997). The development of HR variability in avian embryos may be related to the development of autonomic nervous function (Tazawa *et al.*, 1992) and still remains to be elucidated.

Although the MHR in group III eggs decreased with age the pattern was irregular, for example, the MHR was occasionally higher than that recorded on the preceding day, implying changes over a period of hours. Although the 10-min period MHR may not always accurately represent the mean heart rate on any given incubation day in individual embryos, particularly during the later stages, statistical analysis reveals the underlying trends, despite considerable variation. Longer term measurements of HR may identify other factors influencing the cardiac rhythm of developing avian embryos.

Developmental patterns of MHR

Although the MHR was not determined every day for the same individuals, developmental patterns were evident in data from the 3 age groups, (I, II and III). Until about 60% of the incubation period, the MHR remained at about 185 bpm, independent of $GspH_2O$. It then began to decrease, with embryonic development depending upon $GspH_2O$; HR differed between the 3 groups with different $GspH_2O$. High $GspH_2O$ may be a factor maintaining a high HR, although one embryo with high $GspH_2O$ had a HR of 170 bpm until day 35, its MHR decreased to 114 bpm on the following day and it died before measurement on day 37. High GH_2O had no effect on oxygen uptake of chick embryos (Okuda and Tazawa, 1988; Ar *et al.*, 1991), but increased water loss from the egg, resulting in retardation of embryonic development and high mortality (Okuda and Tazawa, 1988; Meir *et al.*, 1984; Meir and Ar, 1987, 1991, 1996; Ar *et al.*, 1996). In the ostrich, low $GspH_2O$ eggs hatched later than high $GspH_2O$ eggs (Ar *et al.*, 1996). Ostrich embryos may match their chorioallantoic blood flow to their shell GH_2O, such that the G/Q ratio is fixed. Ar *et al.* (1996) showed that ostrich eggs with high and low $GspH_2O$ had a mortality of 21%, compared to 12% in normal (medium) $GspH_2O$ eggs.

HR during the periods 19 to 23, 26 to 30 and 33 to 37 d of incubation was not significantly different between low and medium $GspH_2O$ groups. In chicken eggs, no correlation was found between embryonic HR and eggshell GH_2O which was lowered by applying adhesive PVC tape on the shell surface (Laughlin, 1978). However, in the ostrich eggs, although the low $GspH_2O$ did not decrease the HR relative to medium $GspH_2O$, the developmental pattern over all age groups became different between the 2 $GspH_2O$ groups.

In precocial birds, HR tends to decrease towards the end of prenatal development, except for peafowl embryos which gradually increased HR towards external pipping. Goose embryos decreased HR with development up to about 80% of the incubation period and then increased it towards hatching (Tazawa *et al.*, 1991). The present study shows that in ostrich embryos HR decreases during the last stages of development up to about 90% of incubation, as expected from the general trend in precocial birds. However, HR during the remaining 10%, including prepipping HR and external pipping HR, remains to be determined.

In the hatchery, the humidities of the incubators were adjusted to induce close to normal water loss in most of the eggs, to avoid the adverse effect of excessive water loss on the embryonic development and consequentially on the HR. However, despite this adjustment, the HR of high $GspH_2O$ eggs was high compared to other groups, and it is likely that the humidity of the incubator containing high $GspH_2O$ eggs was not appropriate for most of the eggs. This particular aspect should be investigated.

Allometric relationship with egg mass

The mean egg mass of the 36 eggs used for the present measurement was 1328 ± 163 g. An allometric equation relating HR to egg mass has been determined for data derived from 6 species of domesticated precocial birds at 38°C (Tazawa *et al.*, 1991). The MHR predicted from the allometric equation for the mean egg mass of 1328 g was 184 bpm at 80% of incubation period. The value was corrected to 165 bpm for an incubation temperature of 36·3°C by assuming $Q_{10} = 2$. The average HR measured in the present study for 36 embryos on d 33 and d 34 of incubation (corresponding to 80% and 83% of the incubation period, respectively) was 155 ± 14 and 159 ± 12 bpm, respectively. Taking into consideration the wide

variation in MHR late in incubation, these values are close to the predicted (and corrected) HR.

ACKNOWLEDGEMENTS

We are grateful to Mr Dany Campi, hatchery manager, and Mr Nati Aizik, R and D manager, of Zemach Ostriches for their kind collaboration. Zemach Ostriches kindly allowed us to use their incubated ostrich eggs. This work was supported partially by the Monbusho International Scientific Research Program (Joint Research) awarded to HT (No. 07044110).

REFERENCES

AKIYAMA, R., ONO, H., HÖCHEL, J, PEARSON, J.T. & TAZAWA, H. (1997) Noninvasive determination of instantaneous heart rate in developing avian embryos by means of acoustocardiogram. *Medical & Biological Engineering & Computing*, **35:** 323–327.

AR, A., GIRARD, H. & RODEAU, J.L. (1991) Oxygen uptake and chorioallantoic blood flow changes during acute hypoxia and hyperoxia in the 16 d chicken embryo. *Respiration Physiology*, **83:** 278–295.

AR, A., MEIR, M., AIZIK, A. & CAMPI, D. (1996) Standard values and ranges of ostrich egg parameters as a basis for proper artificial incubation, in: DEEMING, D.C. (Ed.) *Improving our Understanding of Ratites in a Farming Environment*, pp. 131–144. Ratite Conference, Banbury, Oxon.

BURGGREN, W.W., TAZAWA, H. & THOMPSON, D. (1994) Genetic and maternal environmental influences on embryonic physiology: intraspecific variability in avian embryonic heart rates. *Israel Journal of Zoology*, **40:** 351–362.

DEEMING, D.C., AYRES, L. & AYRES, F.J. (1993) Observations on the commercial production of ostrich (*Struthio camelus*) in the United Kingdom: incubation. *Veterinary Record*, **132:** 602–607.

HAQUE, M.A., WATANABE, W., ONO, H., SAKAMOTO, Y. & TAZAWA, H. (1994) Comparisons between invasive and non-invasive determinations of embryonic heart rate in chickens. *Comparative Biochemistry and Physiology*, **108A:** 221–227.

LAUGHLIN, K.F., LUNDY, H. & TAIT, J.A. (1976) Chick embryo heart rate during the last week of incubation: population studies. *British Poultry Science*, **17:** 293–301.

LAUGHLIN, K.F. (1978) The effects of restricted gas exchange on embryonic heart rate. in: PIIPER, J. (Ed.) *Respiratory Function in Birds, Adult and Embryonic*, pp. 298–303. Berlin, Springer- Verlag.

MEIR, M., NIR, A. & AR, A. (1984) Increasing hatchability of turkey eggs by matching incubator humidity to shell conductance of individual eggs. *Poultry Science*, **63:** 1489–1496.

MEIR, M & AR, A. (1987) Dynamic control of incubation conditions to match eggshell conductance variability: suggestions for incubator management. *Turkeys*, **35:** 20–28.

MEIR, M. & AR, A. (1991) Compensation for seasonal changes in eggshell conductance and hatchability of goose eggs by dynamic control of egg water loss. *British Poultry Science*, **32:** 723–732.

MEIR, M. & AR, A. (1996) Artificial increase of eggshell conductance improves hatchability of early laid geese eggs. *British Poultry Science*, **37:** 937–951.

OKUDA, A. & TAZAWA, H. (1988) Gas exchange and development of chicken embryos with widely altered shell conductance from the beginning of incubation. *Respiration Physiology*, **74:** 187–198.

RAHN, H., POTURALSKI, S.A. & PAGANELLI, C.V. (1990) The acoustocardiogram: a noninvasive method for measuring heart rate of avian embryos. *Journal of Applied Physiology*, **69:** 1546–1548.

TAZAWA, H., AR, A., RAHN, H. & PIIPER, J. (1980) Repetitive and simultaneous sampling from the air cell and blood vessels in the chick embryo. *Respiration Physiology*, **39:** 265–272.

TAZAWA, H. (1981*a*) Effect of O_2 and CO_2 in N_2, He and SF_6 on chick embryo blood pressure and heart rate. *Journal of Applied Physiology*, **51:** 1017–1022.

TAZAWA, H. (1981*b*) Measurement of blood pressure of chick embryo with an implanted needle catheter. *Journal of Applied Physiology*, **51:** 1023–1026.

TAZAWA, H., KURODA, O. & WHITTOW, G.C. (1990) Noninvasive determination of embryonic heart rate during hatching in the Brown Noddy (*Anous storidus*). *Auk*, **108:** 594–601.

TAZAWA, H., HIRAGUCHI., T., KURODA, O., TULLETT, S.G. & DEEMING, D.C. (1991) Embryonic heart rate during development of domesticated birds. *Physiological Zoology*, **64:** 1002–1022.

TAZAWA, H., HASHIMOTO, Y. & DOI, K. (1992) Blood pressure and heart rate of chick embryo (*Gallus domesticus*) within the egg: Responses to autonomic drugs. in: HILL, R.B., KUWASAWA, K., MCMAHON, B.R & KURAMOTO, T. (Eds) *Phylogenetic Models in Functional Coupling of the CNS and the Cardiovascular System*, (Basel, Karger), pp. 86–96.

TAZAWA, H., WATANABE, W. & BURGGREN, W.W. (1994) Embryonic heat rate in altricial birds, the pigeon (*Columba domestica*) and the bank swallow (*Riparia riparia*). *Physiology Zoology*, **67:** 1448–1460.

TAZAWA, H. & WHITTOW, G.C. (1994) Embryonic heart rate and oxygen pulse in two procellariiform seabirds, *Diomedea immutabilis* and *Puffinus pacificus*. *Journal of Comparative Physiology B*, **163:** 642–648.

WANG, N., BUTLER, J.P. & BANZETT, R.B. (1990) Gas exchange across avian eggshells oscillates in phase with heartbeat. *Journal of Applied Physiology*, **69:** 1549–1552.