

cultured granulosa cells of the Japanese quail. Melatonin decreased LPS-induced expression of *IL-1 β* , *IL-6*, *IL-8*, and suppressed the nitrite level. On the contrary, melatonin increased the dityrosine level. In addition, melatonin administration increased the viability of LPS-stimulated granulosa cells *in vitro*. These results suggest that melatonin protects cultured granulosa cells from LPS-induced inflammatory and oxidative stress damage and provide evidence that melatonin might have therapeutic utility in ovarian follicle infection in the Japanese quail.

4. Melatonin does not affect progesterone basal secretion but suppresses the luteinizing hormone receptor expression in granulosa cells of the Japanese quail

Whether exposure of granulosa cells of the Japanese quail to melatonin would create changes in progesterone production was determined. For *in vitro* experiments, granulosa cells were isolated from pre-ovulatory follicles (F1–F3) when the F1 follicles were predicted to be either immature or mature (at 3–6 or 18–21 h after oviposition, respectively). Granulosa cells were cultured for 12 h with or without melatonin concentration gradients of 0.0001–100 $\mu\text{g/mL}$, thereby averting luteinizing hormone (LH) stimulation. It was found that melatonin receptor subtypes (*Mel-1a*, *1b*, and *1c*) were expressed in the granulosa cells of the pre-ovulatory F1 follicles. Melatonin suppresses the *LHCGR* mRNA expression at low concentrations in granulosa cells of F1 follicles but does not affect the basal secretion of progesterone in cultured granulosa cells of the F1–F3 follicles. For the *in vivo* experiment, quails received intraperitoneal injection of melatonin (0.67 mg/kg body weight) or mock-vehicle at 3 or 18 h after oviposition, respectively. The birds were decapitated to collect serum 3 h later (at 6 or 21 h after oviposition, respectively). Results shows that melatonin treatment at a low concentration has no influence on the serum progesterone concentration at 6 h post-oviposition, but suppresses progesterone level 21 h after oviposition in the Japanese quail. These results demonstrated that only the low melatonin concentrations had negative effects on progesterone production of the Japanese quail; with the drastically exceeding physiological melatonin used in the anti-inflammatory experiment, no harmful effects were detected under the present situation.

5. General summary

Hypertonic melatonin concentrations protected the bMECs and granulosa cells from the LPS-induced cell damage, and had no harmful effects on progesterone production of the Japanese quail. These findings add some new information to develop succedanea of antibiotics, and therefore may contribute to the improvement of livestock industry and the public health.