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### Endocrinology

maintaining a functionally non-receptive uterine surface with regard to blastocyst attachment. Muc-1 generally reduced in the luminal epithelium during conversion to a receptive state. In this study, Muc-1 mRNA expression was investigated in uterine epithelial cell lines established from  $p53^{-/-}$  adult female mice. The lines possessed estrogen receptor. The lines have been cultured in DMEM/F12 containing fetal calf serum at 10%, supplemented with insulin (10 µg/ml), transferrin (10 µg/ml), cholera toxin (10 ng/ml), selenium (10<sup>8</sup> M), and estradiol-17 $\beta$  (10<sup>-8</sup> M). Muc-1 mRNA expression in the lines was confermed with RT-PCR. Further analysis is under investigation whether the mRNA expression in the lines is affected by steroid hormones.

### SEX REVERSAL BY AROMATASE INHIBITOR TREATMENT IN THE PROTOGYNOUS WRASSE, HALICHOERES TRIMACULATUS

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In protogynous wrasse, social cues stimulate sex change of their behavior, body coloration and gonad, which completes total transformation in a few weeks. The transformation is accompanied with rapid decline of serum estradiol (E2) levels and gradual increase of serum 11-ketotestosterone levels. However, it is still uncertain whether the changes in the steroid levels actually trigger sex change. We report here that a non-steroidal aromatase inhibitor (A1), fadrozole, can stimulate sex change in the threespot wrasse, *Halichoeres trimaculatus*. Adult females were fed with diets containing different amounts of A1 (0, 100 and 500 mg/kg) alone or with estradiol (0 and 100 mg/kg). After two or six weeks, gonads were removed and fixed for standard histological analysis. A1 at concentrations of 100 and 500 mg/kg diet resulted in complete sex reversal of all individuals. They showed typical body coloration of terminal phase males, and had functional testis with all stages of spermatogenesis. Addition of E2 in the same diets prevented fish from changing sex. These results suggest the key role of E2 in both remaining and leaving female sexuality in the wrasse.

### CHARACTERIZATION OF GONADOTROPHS IN THE PARS TUBERALIS OF HAMSTERS

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To clarify characteristics of gonadotrophs (GTH) in the pars tuberalis (PT) of hamsters, *Phodopus sungorus*, we examined the immunoexpression of estrogen receptor (ER $\alpha$ ) and the proliferative activity (BrdU and PCNA) of GTH in the PT, with special references to photoperiod and sex steroid. The exposure to a long-day (LD: 16 h light and 8 h dark) photoperiod increased frequencies of ER $\alpha$  of GTH in the pars distalis (PD) during the postnatal period and exposure to a short-day (SD: 8 hr light and 16 hr dark) declined them. In contrast, frequencies of ER $\alpha$  of GTH in the PT were considerably lower than those in the PD and no difference on frequencies of ER $\alpha$  of GTH in the PT were considerably lower than those in the PD and no difference on frequencies of ER $\alpha$  of GTH in the PT were considerably lower than those in the PD and no difference on frequencies of ER $\alpha$  of GTH in the PT were considerably lower than those in the PD and no difference on frequencies of ER $\alpha$  of GTH in the PT were considerably lower than those in the PD and no difference on frequencies of ER $\alpha$  of GTH in the PT were considerably lower than those in the PD and no difference on frequencies of ER $\alpha$  of GTH was observed in the PT between LD and SD. On the other hand, different photoperiods did not alter the proliferative activity of GTH in both PD and PT. During ontogenesis, the appearance of gonadotropin-immunoreactive cells was later in the PT than in the PD and no ER $\alpha$ -immunoreactive GTH also was detected in the PT.

# EFFECTS OF THE ENDOCRINE DISRUPTOR, BISPHENOL A, ON PLANARIAN SEXUAL REPRODUCTION

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Numerous chemicals released into the environment have been reported to disrupt the development and functions of the endocrine system of wildlife. In mammals, an estrogenic compound, bisphenol A, can easily cross the placenta, get into the fetus and eventually cause abnormalities to the gonads. We investigated the effects of bisphenol A on the sexual reproduction system of the planaria, *Dugesia ryukyuensis*. After ablation at the proximal and distal portions of the pharynx, the fragments were allowed to regenerate in the absence of presence of bisphenol A. In control conditions, all fragments regenerate into normal worms with the female sexual organs like the yolk glands and ovary within 4weeks after ablation. However, in the presence of an optimal concentration (0.01 ppm) of bisphenol A, they regenerate into apparently normal worms but they do not regenerate sexual organs even after 7 weeks. Although little is known about the endocrine system of the planarian, the data suggest that the worms have an endocrine system which play a key role for the formation and/or maturation of sexual organs.

### THE TRANSITION OF FETAL LEYDIG CELLS AND THE DIFFERNTIATION OF ADULT LEYDIG CELLS IN THE POSTNATAL MOUSE TESTIS

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The purpose of this study is to investigate the relationship between fetal Leydig cells (FLC) regression and adult Leydig cells (ALC) differentiation in the mouse testis. We observed the transition of FLC at postnatal Day 0, 3, 5, 8, 11, 13, 14, 35. Both FLC and ALC were marked with histochemical staining of  $\beta\beta$ -hydroxysteroid dehydrogenase ( $\beta\beta$ -HSD). Because FLC are surrounded by basement membranes but ALC are not, we detected laminin, a component of basement membrane, in the postnatal mouse testis. FLC existed in a large population at Day 0, gathered into clusters from Day 5 and were surrounded by delicate basement membranes at Day 11. The clusters came to localize in peripheral regions as testis developed and we found them in the testis even at Day 35. Although  $\beta\beta$ -HSD in FLC remained active, the serum level of testosterone detected using RIA decreased at Day 8. On the other hand, ALC started differentiation at Day 13. The relationship between FLC functional regression and ALC differentiation was discussed on the basis of our results.

### EFFECT OF BRADYKININ ON MMP EXPRESSION IN MOUSE OVARY

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Bradykinin (BK) is a nonapeptide produced by the operation of kallikrein-kinin system, which consists of kallikreins, kinins, kininogens, kallikrein inhibitors, kininase, and kinin receptor. The peptide is a potent mediator of a wide variety of responses in mammalian tissues. BK is also known to play some roles in the reproductive process, such as embryo implantation and follicular contraction at ovulation, uterine contraction at parturition. The effects are mediated by specific receptors, particularly the B2 receptor subtype. Previously, we found that BK was detectable in the ovarian follicular fluid and that the receptor was localized in the granulose cells of all growing follicles. In this study, MMP-20 gene expression found to be induced by BK treatment in mouse ovary culture system. In addition, we isolated a novel type of MMP-20 cDNA clone, which is shorter in length that that reported previously. This report also describes some properties of this unique MMP.

## AN ENDOCRINOLOGICAL STUDY ON THE REPRODUCTION AND HORMONAL CHANGES IN KAGU (RYNOCHETOS JUBATUS)

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The authors tried to obtain reproductive information on the endangered species Kagu, an endemic species in New Caledonia to determine time-wise changes in the concentrations of fecal Testosterone (T), Estradiol-17 $\beta$  (E<sub>2</sub>) and Progesterone (P<sub>4</sub>). They used pairs of Kagu kept at Preservation and Research Center, City of Yokohama, Japan, for the measurement, and VTR recording of the reproductive behavior was also employed. The egg-laying activity was observed from October to May, and, in cases of incubation interruptions, repeated egg-layings were recorded. During this period, T and E<sub>2</sub> showed cycles of rapid increase and decrease within a short time; the amount of E<sub>2</sub> contained in 1 g of feces showed an increase to the level above 1.2 ng/g and decreased promptly within a few days to less than 0.1 ng/g. These rapid hormonal drops coincided with 12 out of 16 eggs. Incubation was carried out by both of the pair but the female incubated 80% of the time. In the male bred individually, the value of P<sub>4</sub> rose to a level equivalent to that of female. At present the authors are engaged in establishing the means of determining the origins of feces by Kagu sex.

### THE ROLES OF THECA CELL LAYER FORMATION DURING FOLLICULOGENESIS IN MOUSE OVARY

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For studies on folliculogenesis, the follicle culture method was designed. The secondary follicles, at diameter of about 120 µm, were isolated from juvenile ovaries by the mechanical method. Individual follicle was cultured in collagen gel containing DMEM supplemented 10% FBS with or without hFSH (11U/ml). During 7 days culture period, the follicles grew in spherical structures. Human FSH stimulated granulosa cell proliferation, and follicle diameter consequently increased to more than 200 µm. Neither antrum formation nor theca cell proliferation was observed. Over 7 days culture, the follicles eventually bursted. In the coculture with dispersed ovarian interstitial cells, follicles were surrounded with interstitial cells, showing the similar structures than those of intact follicles. The origin and role of theca cells on folliculogenesis were discussed.