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Developmental Changes in Expression of Follicle Cell- and Nurse Cell-specific Genes in the Ovaries of *Galleria mellonella*. V.S. Rajaratnam and A.K. Kumaran. Department Biology, Marquette University, Milwaukee, WI 53233.

Oogenesis in insects involves coordinated regulation of gene expression in the germline and somatic cells within the ovarian follicle. To identify some of the genes involved in oogenesis in moths, we isolated 6 different ovary-specific cDNAs from *Galleria mellonella*. After 2 rounds of differential screening of a pharate adult ovarian cDNA library in λgt10 with ovary and male pharate adult cDNAs, 27 clones proved to be ovary-specific. Based on crosshybridization analysis, these clones were grouped into six different classes. We subcloned the cDNA inserts in pGem-3Z and, to date, we have characterized three of these clones. These clones, OV11, N23 and F20, hybridize to 1.1, 2.3 and 2.0 kb transcripts that are present only in the ovary. In addition to these abundant transcripts, F20 hybridized to a less abundant (approximately 25%) 4.0 kb transcript. *In situ* hybridization analysis showed that F20 and N23 are follicle cell- and nurse cell-specific, respectively. Developmental dot blot analysis showed that the transcripts complementary to F20 and OV11 are most abundant in vitellogenic stages and were not detectable in previtellogenic, late chorionic and mature eggs. N23 complementary transcripts were detectable all through oogenesis beginning early before initiation of vitellogenesis, and the transcripts persist in mature eggs and early embryos. The persistence of the nurse cell-specific transcript, N23, in early embryos suggests that it may represent a developmentally significant gene. In its developmental profile and tissue specificity of expression, as well as the size of the transcript, F20 has the predicted properties of the gene coding for the large (74 kDa) follicle cell-specific vitellogenin. Supp. by N.S.F. grant #89-10757.

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26 and 29 Kdal proteins as markers for granulosa cell differentiation from pre- and antral follicles. A. Sanbuissho, G.Y. Lee and E. Anderson, Department of Anatomy and Cellular Biology, Harvard Medical School, Boston MA 02115.

Rat granulosa cell differentiation was studied by gel electrophoretic analysis of [^{35}S]-methionine incorporation into cellular proteins and production of steroid hormones in vitro. Examination of radiolabled proteins provides a qualitative measure of functional differentiation of granulosa cells. Granulosa cells isolated from 15, 25 day old and PMSG primed (27 day old) rats were cultured for 4 days in serum free DMEM/F-12 medium with FSH (0.1-100 ng/ml), FSH plus Δ^4-androsteindione (0.1 μM) or dbcAMP (0.001-1 mM). After 4 days of culture, granulosa cells were incubated with 100 μCi/ml [^{35}S]-methionine for 2 h. FSH stimulated granulosa cells from PMSG-primed rats to produce two distinct protein bands (26 and 29 Kdal) as identified on SDS-PAGE, in a dose-related manner whereas only the highest dose of dbcAMP (1 mM) produced the equivalant bands as that by FSH stimulation. Granulosa cells from 15 day old rats did not respond to any of the above conditions. Those from 25 day old rats responded to FSH plus Δ^4-androsteindione in a dose related manner and to the highest dose of dbcAMP (1 mM). Granulosa cells from PMSG-primed rats secreated the most progesterone and estradiol 17β as compared to those from 15 day and 25 day old rats which suggested to us that granulosa cells from PMSG-primed rats are functionally differentiated cells. Our findings indicate that synthesis of 26 and 29 Kdal proteins are follicle stage-dependent and suggest that these proteins may provide useful markers for granulosa cell differentiation and function. Supported by NIH-HD14574

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Follicle-Specific Protein in the Stick Insect, Carausius morosus (Br.). B. Estridge[1], A. Cecchettini[2], J. Bradley[1], and F. Giorgi[2]. [1]Department of Zoology, Auburn University, Auburn AL 36849 and [2]Department of Biomedicine, University of Pisa, Pisa, Italy.

During vitellogenesis, terminal follicles of the panoistic ovarioles of Carausius morosus sequester two multisubunit vitellogenins (VG) that are synthesized and secreted by the fat body. Separation of egg yolk polypeptides by SDS-PAGE reveals five vitellin (VG-derived) polypeptides with molecular weights ranging from 60 kD to 180 kD and a sixth major polypeptide (A_4) at 93 kD. The absence of detectable A_4 in hemolymph proteins separated by SDS-PAGE and stained with Coomassie brilliant blue suggested that A_4 might be a follicle-specific protein (FSP). In order to test this, a polyclonal antiserum and three monoclonal antibodies were prepared against A_4 obtained by transfer of polypeptides from polyacrylamide gels to nitrocellulose paper. These antibodies were used in immunoblots in conjunction with fluorographs of polyacrylamide gels to analyze the polypeptides synthesized and secreted by fat body, whole staged follicles, and follicle cell layers isolated from staged follicles. Both *in vivo* and *in vitro* data indicate that A_4 is not produced by the fat body. These characteristics identify A_4 as a FSP. *In vivo* incorporation of ^{32}P-orthophosphate into the major egg yolk polypeptides showed that all five VG polypeptides are phosphoproteins. No detectable ^{32}P was incorporated into A_4. Supported by Alabama NSF/EPSCoR Grant #RII-8610669 and the Alabama Agricultural Experiment Station.

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Immunohistochemical Localization of Epidermal Growth Factor (EGF) Receptor in Normal and Polycystic Rat Ovaries. J Yeh, GY Lee and E Anderson, Depts Obstet/Gynecol & Anat/Cell Biol, Harvard Medical School, Boston, MA 02115.

The EGF receptor is a 170 kD polypeptide with tyrosine kinase activity which serves as the receptor for EGF and transforming growth factor-alpha (TGF-a). Recently, we showed that TGF-a mRNA is present in rat granulosa cells (Yeh et al., J Cell Biol 111:368a, 1990). In addition, by immunohistochemistry, we localized TGF-a to granulosa cells and oocytes of non-antral follicles of normal and polycystic rat ovaries (Yeh et al., J Cell Biol 115:267a, 1991). The presumed mechanism by which ovarian TGF-a exerts its effects on granulosa cells is autocrine or paracrine. In this study, we examined the localization of the EGF receptor in normal and polycystic ovaries. Day 25 Sprague-Dawley rats were injected with vehicle or dehydroepiandrosterone for up to 27 days to produce polycystic ovaries. The ovaries were fixed, sectioned and incubated with anti-EGF receptor antibody which recognized the rat peptide (#06-129, UBI, Lake Placid, NY). Sections were stained with a FITC labeled secondary antibody and examined by fluorescent microscopy. We found the following areas were positive for the EGF receptor: (1) granulosa cells of normal ovaries; (2) granulosa cells of polycystic ovaries; and (3) oocytes of non-antral follicles. The theca was negative for EGF receptor. The pattern of immunofluorescence is similar for both TGF-a and the EGF receptor, which implies that TGF-a exerts its actions locally. This pattern of distribution of the EGF receptor confirms the possible role of TGF-a in the stimulation of granulosa cell function in developing follicles and in the cystic follicles of polycystic ovaries. (Supported by NIH HD28584 and HD14574).

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Production of Cortical Granules during Oogenesis in the Mouse. T. Ducibella and P. Duffy, Depts. of OB/GYN (Division of Reproductive-Endocrinology) and Anatomy and Cellular Biology, Tufts University School of Medicine and New England Medical Center, Boston, MA 02111.

At fertilization, cortical granule (CG) enzymes biochemically modify the mammalian egg's zona pellucida, which results in an important block to polyspermy. The increased incidence of polyspermy observed in germinal vesicle (GV) stage mammalian oocytes may be due to a failure to release a sufficient number of CGs. The objectives of this study were to quantify the number of CGs in the cortex during oogenesis and determine the stage of oogenesis at which CGs reach the high density (~ 30 CGs/100 μm^2) found in metaphase II (MII) eggs. Groups of small oocytes (40-49, 50-59, 60-69, and 70-79 μm diameter) were harvested from 12-13 day old mice and 80 μm GV oocytes from 6-8 wk old mice. Oocytes were fixed and stained with *Lens culinaris* agglutinin for CGs which were quantified by computerized image analysis. CG density (the mean number of CGs/100 μm^2 of cortex) increased exponentially from 3 to 34, in 40 μm to 80 μm oocytes, respectively. When oocyte size was accounted for in these groups, the mean number of total CGs in the cortex per oocyte increased from about 300 to 6000 CGs. CG density was also correlated with the stage of GV chromatin organization during follicle development. The 70-79 μm group had less than 50% of the granules (in the cortex) observed in 80 μm GV oocytes or MII eggs. The data indicates a massive production and/or cortical migration of CGs during the last stage of oocyte growth. Therefore, polyspermy in <80 μm GV stage oocytes may be attributable to low CG number in the cortex. However, full grown GV stage oocytes are characterized by an adequate number of CGs (and reduced activation ability as shown previously by our lab). Support: HD 24191.

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A Germ Cell Protein in the Indianmeal Moth, Plodia interpunctella. P.D. Shirk, G. Zimowska, and E.F. Beckemeyer, USDA ARS, Insect Attractants, Behavior, and Basic Biology Res. Lab., Gainesville, FL 32608.

A protein found primarily in germ cells was discovered in the Indianmeal moth. Germ cell protein (GCP) was purified from embryos and has a molecular weight of 22,000. A GCP monospecific polyclonal antiserum was raised and was used to localize GCP in embryos and germ cells. Using immuno-gold localization, GCP was observed in embryonic cytoplasm but not in yolk spheres. Oocytes contain large amounts of GCP which remains throughout embryogenesis. The amount of GCP decreases rapidly following hatching of first instar larvae as yolk sack materials are digested. GCP was present in germ cells, both female and male, during all stages of development. Using immuno-fluorescent staining in conjunction with Confocal microscopy, GCP was detected in the cytoplasm and nuclei but not in the nucleoli of germ cells. GCP was observed in the cytoplasm and nuclei of nurse cells and the cytoplasm of oocytes. GCP appeared transiently in the nuclei of ovarian sheath cells of newly pupated females, and in the cells of the upper vas deferens during the period of sperm release from testes. In addition to germ cells, GCP was detected constitutively in oenocytes throughout the pupal and adult stages. GCP function has not been determined.