Female Reproduction III: Ovulation, Fertilization

671 PHYSIOLOGY

DEVELOPMENT OF THE LAPAROSCOPIC TECHNIQUE FOR IN VIVO COLLECTION OF FOLLICULAR OOCYTES IN SHEEP (29813). Alvaro Mondaca and L. E. Casida. Endocrine Research Unit, Michigan State University, East Lansing, MI 48824

From preliminary studies in our laboratory, we have found that oocytes collected from sheep ovaries removed at the time of slaughter, can be matured in vitro. The purpose of this study was to establish a technique for the in vivo collection of follicular oocytes for in vitro studies, with a minimum of surgical trauma.

Ventral laparoscopy was performed on ewes under general anesthesia. A 135-degree pediatric laparoscope was inserted through a 6 mm cannula, placed through a 1 cm skin incision and muscle puncture mid-ventrally 1 to 2 cm anterior to the mammae. The laparoscope was secured with a tourniquet clamp and ring stand. Ovaries were held stationary with a pair of laparoscopic grasping forceps placed 5 to 6 cm lateral to the laparoscope. Ovarian follicles were aspirated with a 2.5 ga, 11 cm needle inserted through an 18 ga. cannula placed along the mid-line at the posterior border of the mammae and equipped with a 2, 5 ml syringe.

A total of 200 laparoscopic examinations have been performed on 25 ewes with examination as often as every other day. Preliminary studies have involved 21 follicles of various size aspirated with 6 ova recovered for a 28% recovery rate.

672 PHARMACOLOGY

CHROMOSOME ABNORMALITIES OF INTRAUTERAL RABBIT EMBRYOS INDUCED BY SUPEROVULATION. S. Fujimoto, S. Arisa* and W. R. Dukelow, Department of Obstetrics and Gynecology, School of Medicine, Hokkaido University, Sapporo, 060 Japan.

Chromosomal imbalance is related to some developmental anomalies and wastage in utero. Cytogenetic data of early intrauteral embryos is more informative than those obtained from in utero blastocysts.

Superovulation was induced in 6 mature virgin New Zealand White rabbits by a regime of 100 i.u. PMS (im) and 100 i.u. HCG (iv) 4 days later. Nine females were controls. All animals were sacrificed on Day 2 (mating = Day 0). The 8 to 16 cell embryos were cultured with 0.08% Colcemid for 4 to 5 hours at 37°C. The karyotypes were determined for each embryo by the double fixation method (Fujimoto, et al., 1974).

Seventeen chromosomally abnormal cases were observed among the 86 superovulated embryos. They were 9 haploids, 4 triploids, one tetraploid, 5 trisomies, one double trisomy and one mosaic (19.8%). In controls, there were 6 abnormals out of 60 embryos (10.0%).

673 PHYSIOLOGY

ELECTRON PROBE MICROANALYSIS OF PICOLITER SAMPLES OF RABBIT BLASTOCOELIC FLUID. Raymond M. Borland*, John D. Biggers* and Claude Lechene*. (Spon. J.E.Pappenheimer). Dept. of Physiology, Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School, Boston, Mass. 02115

Electron probe microanalysis (Lechene, 1970, 1974) was used to determine the concentration of elements in microdroplets (~1nl) of New Zealand rabbit blastocoelic fluid. Blastocysts (110,115 and 149 hrs p.c.) were flushed from the uteri and samples of fluid were micropunctured from the blastocoele. Microdroplets were placed on a beryllium support, freeze dried under vacuum, and counted for 7 elements with a Cameca MS/46 microprobe. The mean concentrations (±SEM)(mM/l) are:

Element	Hours post-coitus		
	110	135	150
Sodium	139 ± 3.2	133 ± 4.1	146 ± 2.5
Chloride	78.9 ± 2.8	78.3 ± 1.9	94.3 ± 1.8
Potassium	7.11 ± 0.29	10.2 ± 0.31	10.1 ± 0.36
Calcium	0.974 ± 0.056	0.991 ± 0.13	1.93 ± 0.13
Magnesium	0.115 ± 0.023	0.0/ml ± 0.009	0.220 ± 0.029
Sulphur	5.65 ± 0.47	2.87 ± 0.28	3.02 ± 0.18
Phosphorus	2.79 ± 0.22	0.299 ± 0.028	0.221 ± 0.029

These elements may be important in creating a microenvironment for differentiation of the inner cell mass and in the accumulation of fluid in the blastocoele. (Supported by the Rockefeller Foundation, RF#/0060, The Ford Foundation, 720-0369, the NIH Fetal Program,) PO1 HD-06644-03, and the Program Project Grant of NIH,) PO1 HD-06916-01A1.)

674 PHYSIOLOGY

HORMONAL AND REGIONAL OVIDUCTAL INFLUENCES ON EARLY EMBRYO DEVELOPMENT. R.L.Stone*and C.E. Hammer. Div. Reprod. Biol., Dept. Ob.-Gyn., Univ. of Va. Med.Sch. Charlottesville, Va. 22903.

Pronuclear rabbit embryos were transferred to the oviducts of 1-day pseudopregnant(PSP)and 10-day PSP temporary recipients(TRs). Transferred embryos were incubated 50 hr. in whole oviducts with no ligation, oviducts with uterotubal junction ligation (UTJLO), or in ampullae with ampullar-isthmic ligation(AILO). Regardless of tubal restrictions or synchrony of the TRs, 67-79% of the embryos recovered were normal-appearing morulae. Retransfer of the morulae to 2.5 day PSP uterine recipients showed fewer implants(6%) from morulae of the 10-day PSP UTJLO than of the 1-day PSP UTJLO (32%), 1-day PSP AILO(13%),or 10-day PSP AILO(31%). Pronuclear embryos restricted in UTJLO or AILO in 21-day ovariectomized(OX) TRs developed normally to morulae. Only 11% and 13%,respectively, implanted upon retransfer to 2.5-day PSP uteri.Embryos recovered from AILO of OX oestrogen-treated TRs had developed more slowly than those of all other treatments. There were no implants from AILO morulae retransferred to uteri. Progesterone treatment of OX TRs restores normal conditions for embryo development. These data implicate regional and hormonal effects of the rabbit oviduct on early embryo development. (Supported by World Health Organization).

675 PATHOLOGY

EFFECTS OF POLYCHLORINATED BIPHENYLS ON REPRODUCTION IN THE PRIMATE. D.A. Forsgetti* and J.R. Allen. Univ. of Wisconsin, Madison, WI 53706

Adult male and female rhesus monkeys were fed diets containing polychlorinated biphenyls (PCBs-Aroclor 1248, Monsanto Co., Inc.) at levels equal to and one-half that permitted in certain foods destined for human consumption (2.5 and 5 ppm) for 52 weeks. During this period the females on the lower level consumed 182 mg while those on the higher level ingested 364 mg. The males which received only the higher level of PCB consumed 183 mg of PCB. Within 1 to 2 months the females had periorbital edema, alopecia, erythema, and acneform lesions involving the face and neck which persisted throughout the experiment. Even though the males consumed more PCB, they exhibited only moderate periorbital edema and erythema. After having been on the PCB diets for 6 months the females were bred with control males and the experimental males bred with control females. Following lactings only 12.5% of the 5 ppm group and 33.3% of the 2.5 ppm group were pregnant as compared to 90% in a control group of similar size. The conception rate of females bred to PCB males was equally as great as that for animals bred to control males. Throughout the experiment the females that consumed PCBs had higher levels of urinary ketosteroids and a decided increase in length of menses and amount of menstrual bleeding. (Supported in part by U.S. Public Health Service grants 1S 00472, RR-00130, RR-00167 from NIH, and the University of Wisconsin Sea Grant Program.)

676 PHYSIOLOGY

L-10492 AND L-10503, NON-STEROIDAL AND NON-HORMONAL ANTI-FERTILITY AGENTS. Leonard J. Lerner, Giulio Gallimi*, Maria Carla Moore* and Anedeo Omodei-Sale Lepetit Research Laboratories, Milan, Italy 20139.

L-10492, (2-(3-Methoxyphenyl)-3H-naftrindol(3,1-a)isoindole and L-10503 (2-(3-Methoxyphenyl)-3,4-dihydro-s-triazole [5,1-a]isoquinoline were administered in subcutaneous regimens to pregnant Sprague Dawley rats (R) and Syrian hamsters (H). Uteri were examined at autopsy on gestation day 16 (R) or 14 (H) for live and dead fetuses and implantation sites. Both compounds terminated pregnancy at sub-toxic doses in single and multiple dose regimens at various stages of gestation. Smaller doses were required when administered on days 8-10 (R) or 6-8 (H). Smaller single doses were required on days 7 or 8 (R) and 4 or 5 (H). R required smaller doses than H. L-10492 was 3-8 times as potent as L-10503 with multiple and 6-5 times as potent with single injections in R. In H, L-10503 was 2-4 times more potent than L-10492 in multiple and 8-15 times as potent in single injections. L-10503 was also more potent in mice, rabbits and dogs. Both compounds were 100% effective in all species studied. They have no hormonal, anti-hormonal, cardiovascular or CNS activities. The LD_{50} for each compound is 100-1000 times the 100% antifertility dose. Both compounds are orally active but at much higher doses.

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