



AN ATTEMPT TO DEMONSTRATE HYDROXYSTEROID-DEHYDROGENASE ENZYMES IN THE RAT OVARY AT THE ELECTRON-MICROSCOPICAL LEVEL

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► To cite this version:

A. Samuel. AN ATTEMPT TO DEMONSTRATE HYDROXYSTEROID-DEHYDROGENASE ENZYMES IN THE RAT OVARY AT THE ELECTRON-MICROSCOPICAL LEVEL. *Annales de Biologie Animale, Biochimie, Biophysique*, 1976, 16 (2), pp.159-160. <hal-00897028>

HAL Id: hal-00897028

<https://hal.science/hal-00897028v1>

Submitted on 11 May 2020

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JOINT MEETING

ENGLISH SOCIETY FOR THE STUDY OF FERTILITY

SOCIÉTÉ FRANÇAISE POUR L'ÉTUDE DE LA STÉRILITÉ ET DE LA FÉCONDITÉ

TOURS, NOUZILLY, 24-26 MARS 1975

ABSTRACTS

OVAIRE ET ŒUF

MIGRATORY PHASE OF GERM CELLS AND SEXUAL DIFFERENTIATION OF THE GONAD IN SHEEP EMBRYO. — P. MAULÉON. *I. N. R. A., Nouzilly (France)*.

The migratory intragonadal pathway of the germ cells followed by selective phosphatase cytoplasmic affinity clarifies and allows for an earlier identification of the sex gonads.

Three periods are distinguished and the time defined : hind-gut, mesentery and settlement stages at 17-18-19 days, 20-21-22 days, 23 to 29 days of age respectively.

At 32 days, the gonadal locations of the germ cells change with sex :

- the presumed male : a marked decrease of germ cells in the peripheral zone and those centrally disposed increasing in numbers ; interstitial tissue organization around clear cords,
- the presumed female : a marked increase of germ cells in the cortex and medullary compact cords with a high phosphatase activity.

A differential migratory pattern of the germ cells into the gonad resembles primary sex differentiation.

AN ATTEMPT TO DEMONSTRATE HYDROXYSTEROID-DEHYDROGENASE ENZYMES IN THE RAT OVARY AT THE ELECTRON-MICROSCOPICAL LEVEL. — A. SAMUEL. *University of Cambridge (G. B.)*.

Ovaries from rats between 10 and 18 days pregnant were fixed for 1 hr in 1 p. 100 glutaraldehyde : 4 p. 100 formaldehyde in 0.1 M *tris*/HCl buffer (pH 7.4). 150 μ frozen sections were incubated with either DHA, oestrone, testosterone or 20 α -dihydroprogesterone and NAD in

buffer. Thiocarbamyl-nitro-BT was used as the reduction indicator. Incubation was carried out at 15°C for 2 hrs and the tissues rinsed in buffer and post-fixed in 1 p. 100 osmium tetroxide in buffer for 1 hr. They were processed for electron-microscopy and thin sections stained with lead citrate. HSD enzymes were localised by precipitation of electron-dense osmium compounds.

DICHOTOMY OF RESPONSE TO HCG BETWEEN THE GRAAFIAN FOLLICLE AND OOCYTE IN PIGS. — R. H. F. HUNTER. *University of Edinburgh (Scotland).*

Recent experiments on the morphology and steroidogenic potential of the Graafian follicle, particularly those conducted *in vitro*, give little indication of the functional status of the oocyte. In studies on maturation of the follicle and oocyte in pigs, we have observed that if ovulation is induced with an injection of HCG in the early follicular phase of the oestrous cycle, many primary oocytes are ovulated. These precociously liberated oocytes do not exhibit a zona reaction, but invariably become highly polyspermic when confronted by capacitated spermatozoa. This dichotomy of response to HCG between the follicle and its oocyte will be discussed.

ULTRASTRUCTURAL OBSERVATIONS ON THE FORMATION AND DEVELOPMENT OF PRONUCLEI IN POLYSPERMIC PIG EGGS. — D. SZÖLLÖSI, *I. N. R. A., Jouy en Josas (France)*, R. H. F. HUNTER, *University of Edinburgh (Scotland).*

Studies on ultrastructural events during fertilization in mammals have been largely confined to laboratory species, although we have published an extensive report on the domestic pig. Using a system that induces varying degrees of polyspermic penetration, ranging from disperm to > 20 vitelline sperm, we have examined the formation of pronuclei and the fate of the male elements. EM features of these eggs will be presented, with emphasis on decondensation of the nuclear chromatin and apparent anomalies of the male pronuclei. Observations will also be included on structural abnormalities in the midpiece of spermatozoa from the fertile boar used in these studies.

STORAGE OF COW EGGS AT ROOM TEMPERATURE AND LOW TEMPERATURES. — A. O. TROUNSON, S. M. WILLADSEN, L. E. A. ROWSON. *Cambridge (G. B.).*

The storage of cow eggs at room temperature (19-21°C) and after cooling to low temperature (0-7.5°C) was examined in two experiments. Morulae (Day 5) and 8-celled eggs (Day 3) were stored in PBS (Dulbecco Phosphate Buffer) or TCM 199 (Tissue Culture Medium 199) for 1-2 hr or 6-7 hr at 19-21°. After storage, eggs were transferred to the ligated rabbit oviduct for 48 hr (Day 5) or 96 hr (Day 3). Storage in PBS resulted in a higher proportion of apparently normal embryos than storage in TCM 199, particularly for Day 3 eggs. Cooling of morulae (Day 5 and Day 6) to temperatures of 0°, 2°, 5° and 7.5° for 2 min, 30 min and 24 hr resulted in a high proportion of degenerate eggs. These findings will be discussed in relation to the recent attempts to freeze cow eggs.