

## A NEW CELLULAR SOURCE FOR ESTABLISHMENT OF THE KARYOTYPE IN THE SOW

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In our former histological studies, a particular mitotic activity was noted in the cells of the corona radiata, cumulus oophorus and granulosa of the Graafian follicles. The observation suggested this tissue as a possible cellular source for assessing the karyotype of the sow. No mention of it in this context has, so far, been made in any data known to us.

In the present investigations, we used as biological material the liquor folliculi of Graafian follicles which presented on the gonadal surface of some slaughtered sows.

Using a 10-ml syringe fitted with an intradermal needle, the liquor was withdrawn from the follicle. This procedure was repeated for all the follicles on both ovaries of the tested sow, which yielded a sufficient quantity (4 to 5 ml) of liquor and cells. Mechanical aspiration loosened the ovum as well as its corona radiata and some cell plaques from the granulosa and cumulus layers. All this material was then drawn into the syringe together with the liquor folliculi.

As soon as this material was obtained, the contents of the syringe were ejected into a vial for laboratory culture of the tissue.

A dose of 0.2 ml colchicin and 1 ml calf serum was added to the liquor in the vial, which was then tightly closed with a rubber plug and incubated in a thermos containing water at 37° C.

The liquor folliculi itself acted as nutrient medium. The incubation lasted 1 or 1½ hr, as recommended by McFeely (1966) for the blastocyst of the sow, and by Shaver & Carr (1967) for the blastocyst of the rabbit.

Transport of the incubated material to the laboratory took some 45 min. On arrival, the vial was immediately introduced into a thermostatically controlled water bath, to complete the incubation time. Some of the samples were left in the thermos throughout the incubation in summer as the water temperature remained sufficiently constant, demonstrating that the thermos can replace other forms of portable incubator.

At the end of the incubation time, the vial was removed from the water bath and its contents were transferred to another vial. This was centrifuged, the supernatant eliminated and 5 ml hypotonic solution (tris-sodium-citrate 0.85%) at 37° C was added to the cellular sediment. Incubation was continued for another 20 min. After re-centrifuging, the samples were fixed in glacial acetic acid and methyl alcohol 1:3.

Each sample was subjected to six changes of fixative: the first fixation was achieved in 30 min, the others in only 15 min. The material was stained with Giemsa, the slides remaining in the stain as long as 45 min.

After being washed in tap water, the samples were dried, mounted and scanned under the microscope at a magnification of  $\times 20$ , to identify the metaphase plates.

In addition to the cellular material procured from the slaughter-house, one sample of liquor was obtained at laparotomy from the follicles of a living sow, in the pro-oestrous phase.

In an attempt to intensify the degree of cellular diffusion, hyaluronidase or Versene were at first introduced into the media, but the results were unconvincing.

Activation of the mitotic process was also attempted, by adding Gonacor (indigenous HCG) to the medium. But, while the samples treated with calf serum yielded a mean of thirty to forty metaphase plates on a slide, only three to four metaphase plates were obtained with the Gonacor.

Our method can be used in a variety of situations, for instance to determine the karyotype of individuals which have been pre-irradiated or treated with drugs. This can be achieved at laparotomy, during the pro-oestrous phase, when liquor folliculi can be withdrawn from the exteriorized gonads and post-operative treatment started at once.

On the basis of these experiments, we conclude that the karyotype of the sow can be determined by using the liquor folliculi and cells detached from the granulosa, the cumulus, or the corona radiata of the follicles in the course of aspiration of the liquor folliculi.

#### REFERENCES

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