A KARYOMETRIC STUDY OF EPITHELIAL CELLS LINING THE GLANDS OF THE BOVINE ENDOMETRIUM

R. H. SCHULTZ, H. B. BURCALOW, M. L. FAHNING,
E. F. GRAHAM AND A. F. WEBER

*Departments of Animal Science, Veterinary Obstetrics and Gynecology,
and Veterinary Anatomy, University of Minnesota, St. Paul*

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Height measurements of surface and glandular epithelium, cell to nucleus length ratios and glandular lumen sizes have been employed for evaluating the endometrial activity of the uterus in the cow (Asdell, DeAlba & Roberts, 1948; Dziuk, Donker, Nichols & Peterson, 1958; Foley & Reece, 1948; Weeth & Herman, 1952; Johnson, 1965). These techniques, however, have not been completely satisfactory. For example, epithelial height measurements were reported to vary greatly in areas of tissue closely adjacent to one another (Dziuk *et al.*, 1958). Johnson (1965) found that epithelial height varied between and within periods of the oestrous cycle to such an extent that this measurement was not valid for estimating the stage of the cycle.

Hultquist (1959) described a technique of karyometry used on β -cells of the islets of Langerhans in rats. Since the nuclear size of the β -cells was found to be altered by starvation (Hultquist, 1962), the author suggested that these alterations reflected changes in cellular activity. This technique was, therefore, selected as potentially effective for evaluating uterine endometrial activity. The object of this study was to determine karyometric differences in the endometrial gland cells from ovariectomized and ovariectomized hormonally-treated cows.

Twenty-seven, adult, reproductively normal, Holstein cows were used in this study. All cows were ovariectomized on Day 10 of the oestrous cycle. Two days later the animals were placed in one of three treatment groups. Each group consisted of nine animals. Group I received 100 mg of progesterone suspended in corn oil. Group II received 3 mg oestradiol-17 β suspended in corn oil. Group III served as the control animals and received 5 ml of corn oil. All treatments were administered subcutaneously for 3 consecutive days. On the 4th day, the cows were slaughtered and the endometrial tissues obtained and placed in 10% formalin until the time of histological preparation. The tissue specimens were cut at a thickness of 7 μ and stained with haematoxylin and eosin using routine histological techniques.

Photomicrographs were taken of the glandular areas of the endometrium using a Leitz Panphot photomicrographic apparatus (Firma Leitz, Wetzlar,

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West Germany). The photomicrographs were taken at a magnification of $\times 980$, using an oil immersion objective with an exposure time of 0.1 sec and a light meter setting of 22. Kodak Pantomic X film (Eastman Kodak Co., Rochester, New York) was used. A series of eleven pictures was taken of each specimen. The photo-microscope was focused on a different glandular area in the tissue for each exposure in order to obtain representative samples of epithelial cells. Only cells lining the basal area of the glands were used, to avoid the possibility of variation in cells located in different regions of the glands.

The developed film was placed in a filmstrip projector and the best ten of eleven pictures were projected against a screen at a given distance to obtain uniform magnification, resulting in a final total magnification of $\times 10,000$. The outlines of ten 'normal' projected nuclei in each section were sketched on white paper, and the surface areas of the nuclei were measured using a compensatory polar planimeter (Keuffel and Esser Co., New York, Number 62005). This provided a total of 100 nuclear measurements from each of twenty-seven specimens.

TABLE 1
MEAN AND STANDARD ERROR (S.E.) VALUES OF NUCLEAR SIZE
MEASUREMENTS (SQUARE MICRONS)

<i>Control</i>		<i>Oestradiol</i>		<i>Progesterone</i>	
<i>Mean</i>	<i>S.E.</i>	<i>Mean</i>	<i>S.E.</i>	<i>Mean</i>	<i>S.E.</i>
29.192	± 0.153	31.196	± 0.175	33.586	± 0.168

'Normal' nuclei were defined for this study as those that were well in focus and did not appear pycnotic. Nuclear types avoided were those that appeared very compact and stained unusually dark or those that demonstrated extreme shapes and sizes in relation to adjacent nuclei. Since the purpose of the study was to compare nuclear size differences between the treatment groups, only nuclei which appeared to have been sectioned through the centre were selected. In an attempt to provide true comparisons, only the largest and most spherical nuclei were sketched and measured. Groups were compared on the basis that all measurements were representative of the same cross-sectional area of the nuclei.

The data collected using the karyometric technique were then subjected to statistical analysis. The mean and standard error were calculated for each group. Differences between groups were determined using the Student-Newman-Keuls Multiple Range test (Steel & Torrie, 1960). The results are shown in Table 1.

Analysis of variance revealed a highly significant difference ($P < 0.01$) in nuclear size between the groups. This difference is attributed to the influence of the steroid hormones on the nuclei of the epithelial cells lining the mucous glands of the endometrium. The Student-Newman-Keuls Multiple Range test demonstrated that a highly significant difference in nuclear size existed between

the control group and the hormonally-treated groups and also between the two hormonally-treated groups.

The data compiled in this study indicate that the nuclei of epithelial cells lining the mucous glands of the bovine endometrium respond, directly or indirectly, to hormonal influences by an increase in size which can be detected through the use of karyometry. Assuming that the hormones used in this study caused an increase in cell-secretion rate, these data suggest that an increase in nuclear size may be associated with increased cell-secretion rate. They further suggest that karyometry may prove to be a technique with an increasingly important rôle to play in future histological research.

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