Weight of ovaries

Group	No. of animals	Evolution (days)	Mean (mg/100 g body weight)	Standard error
Control	10		19.52	± 2.45
HWD	10	10–14	18.86	± 2.21
CWD*	10	10–14	20.80	± 3.32

*The half weight of both ovaries was taken into account for each animal.

were more scarce in the CWD than in normal animals and distributed either in small and medium nodes or sparsely in the ovarian stróma. The other abnormalities were shrinked spindle-shaped cells showing a greater than normal nuclear cytoplasm relationship. A gross accumulation of granulated inclusions stained with PAS, Sudan Black B and Long Ziehl-Neelsen techniques (like lipofuscin pigment) appeared to be contained, both intra and extracellularly, at least in some areas of the ovaries.

In the hemicastrated animals no significant weight change was found in the remaining ovary as compared with both their own control and those of the control water deprived animals. (Table). That is, water depletion was shown to inhibit completely the ovarian compensatory hypertrophy in spite of the reported increase in gonadotrophin release. We assume this to be related to the histologic changes that dehydration produces in the ovaries.

Resumen. En ratas hemicastradas deshidratadas no se observa la hipertrofia compensadora del ovario remanente. Se sugiere que ello pueda estar relacionado con los cambios histológicos del ovario deshidratado.

I.M. Libermann, A. Capano,
J.T. Otegui and O. Botero-Correa

Laboratory of Water and Salt Metabolism and Laboratory of Neuroendocrinology, Department of Physiopathology, Hospital de Clinicas, Montevideo (Uruguay), 11 May 1971.

Plasma Prolactin Levels in Fetal Sheep

Radioimmunoassays for ovine prolactin have been developed in recent years[1–3] permitting the measurement of the levels of prolactin in the plasma of sheep. In this paper we report the development of a double antibody radioimmunoassay for ovine proclactin and its application in determining the prolactin levels in the plasma of fetal sheep.

Methods. The development of the rabbit antiovine prolactin serum used in the radioimmunossay has been previously described by us[4]. Ovine prolactin standard (L3458B, 35 IU/mg, the kind gift of Dr. C.H.Li) was iodinated with ^{131}I by the method of Greenwood et al.[5]. Because the labeling procedure caused some damage to the prolactin it was passed through a Sephadex G-100 superfine column (0.9 × 27 cm), equilibrated and eluted with an 0.08 *M* barbital-acetate buffer, pH 8.6, before being used in the assay. Figure 1 shows a representative elution profile, whose individual tubes were counted on a partially-shielded gamma-sensitive scintillation detector coupled with a scaler. The material in the major peak (fractions 28–30) was diluted with a diluent made by adding bovine serum albumin (Fraction V) to the barbital-acetate buffer to a concentration of 0.5%. 200 µl of the diluted material contained 4000–5000 cpm when counted in a Nuclear Chicago Model 4216 Well Gamma Counter. For the assay, 200 µl of the ^{131}I-labeled ovine prolactin was added to glass culture tubes (13 × 100 mm) to which was added 200 µl of the rabbit antiovine prolactin serum diluted 1:18,000 with diluent. Data for the standard curve were obtained by adding 100 µl of ovine prolactin solutions in concentrations ranging from 0 to 50 ng/ml in diluent. Plasma was also assayed in 100 µl aliquots. Each concentration of standard and each sample was run in triplicate. The tubes were incubated at 4 °C for 5 days at which time 100 µl of 1:10 dilution of goat anti-rabbit γ-globulin serum and 100 µl of a 1:20 dilution of normal rabbit serum were added to each tube. The tubes were incubated for another 6 to 16 h, centrifuged, the supernatants aspirated off, and the precipitates counted in a Nuclear Chicago Model 4216 Well Gamma Counter. The results are expressed as a percentage of the number of counts in the precipitate of the zero prolactin tubes (referred to as percentage of zero binding). Figure 2 shows a representative standard curve and the parallel curves obtained by diluting two samples of wether plasma.

Blood samples were obtained from 11 white-faced range sheep fetuses between 60 and 138 days of fetal age. The fetuses were taken while the ewes were under pentobarbital anesthesia, their hearts removed quickly for other experiments, and the blood sample obtained, in a heparinized syringe, from the aorta.

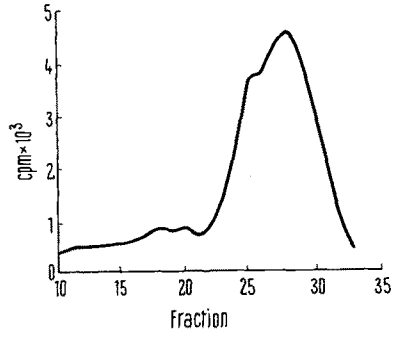

Fig. 1. Elution profile of ^{131}I-labeled ovine prolactin from a Sephadex G-100 superfine column (0.9 × 27 cm). Fraction volume approximately 0.27 ml; 2.0 ml of eluate was collected before the fractions.

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2 G. D. Bryant and F. C. Greenwood, Biochem. J. *109*, 831 (1968).
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4 W. H. Moger and I. I. Geschwind, Gen. comp. Endocrin. *14*, 152 (1970).

Specificity of the assay was determined by allowing other ovine and bovine pituitary hormones to compete with the radioiodinated ovine prolactin for binding by the antiserum. The pituitary preparations employed were ovine and bovine growth hormones, NIH-GH-S9 and NIH-GH-B14, both obtained through the courtesy of the Pituitary Hormone Distribution Program of the Endocrinology Study Section of the National Institutes of Health; ovine LH, IV 28BP, and ovine FSH, G423C, the kind gifts of Dr. HAROLD PAPKOFF; bovine TSH, CCD-V-3, kindly provided by Dr. JOHN PIERCE; and bovine prolactin, NIH-P-B3, also from the Pituitary Hormone Distribution Program. The activity of the bovine prolactin is given as 24.1 IU/mg, while the prolactin activity in the growth hormone preparations is stated to be a maximum of 0.66 IU/mg for the ovine preparation (S9) and of 0.42 IU/mg for the bovine preparation (B14). The results of this testing program are reported in Table I, and indicate: that the bovine prolactin preparation is equipotent to the standard ovine prolactin preparation; that both growth hormone preparations cross-react in this assay to an extent of slightly less than 0.8% (i.e., less than 0.28 IU/mg) and that the slope of the displacement curve is essentially the same as that of the standard, both of which, in conjunction with the bioassay data reported above, suggest that what is being measured is a prolactin contaminant in the growth hormone preparations; and that the 3 highly purified glycoprotein preparations show no competition at the highest levels at which they were tested – 1 μg.

Results and iscussion. The fetal sheep plasma prolactin levels, as a function of age, are listed in Table II. Prolactin was detected at the limit of the assay's sensitivity (3 ng/ml), in one fetus 72 days of fetal age. Substantial amounts of prolactin, however, were found only in fetuses 122 and 138 days of fetal age.

STOKES and BODA[6] could not detect prolactin-containing cells in fetal sheep pituitaries by immunofluorescence until the fetuses were between 80 and 88 days of age. In our series, one blood sample from a 72-day fetus had a barely detectable prolactin level, and we first found substantial amounts of prolactin in plasmas from twin fetuses 122 days of fetal age. This corresponds to the age (125 days) at which Herlant's stain was reported by STOKES and BODA[6] to first differentiate 2 types of acidophils in the pituitary. If it is assumed that at this fetal age there has been no sudden marked decrease in the distribution volume for prolactin, or marked increase in the half-life of the circulating hormone, then these data would indicate that the sheep pituitary begins to secrete substantial amounts of prolactin between 110 and 122 days of fetal age, that is, early in the last trimester of gestation.

Table I. Cross-reaction of pituitary hormones in the ovine prolactin radioimmunoassay[a]

Preparation	Source	Level tested (ng/ml)	Ovine prolactin equivalent (ng/ml)
GH-S9	Ovine	4000	31
		2000	15
		1000	9
GH-B14	Bovine	1000	8
		500	<4
		250	<4
LH-IV28BP	Ovine	1000	<4
		500	<4
		250	<4
FSH-G423C	Ovine	1000	<4
		500	<4
		250	<4
TSH-CCD-V-3	Bovine	1000	<4
		500	<4
		250	<4
Prolactin-B3	Bovine	64	64
		32	33
		16	16.4

[a] In these assays the sensitivity was 4 ng/ml.

Table II. Plasma prolactin levels in fetal sheep

Ewe No.	Sex of fetus	Fetal age, in days	Plasma prolactin (ng/ml)
4-081	?	60	<3
5-251	♂	72	3
1-034	♂	74	<3
4-259	♂	76	<3
	♀	76	<3
3-166	Pooled (1 ♂ 1 ♀)	110	<3
3-403	♂	122	54
	♀	122	12
5-108	♂	138	70
	♀	138	5

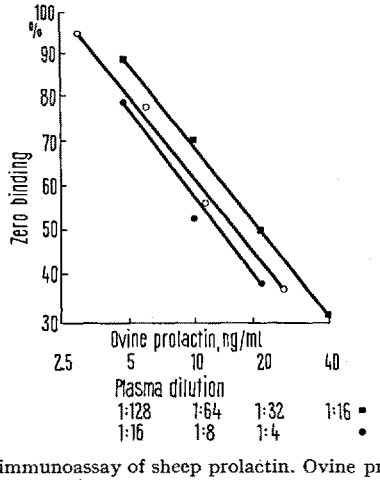

Fig. 2. Radioimmunoassay of sheep prolactin. Ovine prolactin standard (○); wether No. 401 plasma (■) diluted 1:16, 1:32, 1:64 and 1:128; and wether No. 403 plasma (●) diluted 1:4, 1:8 and 1:16.

Zusammenfassung. Entwicklung und Spezifität eines radioimmunologischen Verfahrens für Schaf-Prolaktin. Zirkulierende Prolaktinmengen wurden in Fetussen bestimmt und vom 110. bis zum 122. Tage bedeutende Prolaktinmengen festgestellt.

W.H. MOGER[7] and I.I. GESCHWIND[8]

Department of Animal Science, University of California, Davis (California 95616, USA), 18 June 1971.

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[7] NIH Pre-doctoral Fellow (GM-41356). Present address: Department of Physiology, University of Western Ontario, London (Ontario, Canada).

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