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OVARIAN PROGESTINS IN MASAI GIRAFFE (*GIRAFFA CAMELOPARDALIS*)

S. GOMBE AND F. I. B. KAYANJA*

Department of Animal Physiology, University of Nairobi, Kenya, and
**Department of Veterinary Anatomy, Makerere University, Uganda*

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Summary. Gonadal progestins from fetal, juvenile, pregnant and non-pregnant Masai giraffe (*Giraffa camelopardalis*) were extracted, purified by TLC and measured by GLC or by competitive protein-binding assay. Progesterone was found in fetal ovaries and in a fibrotic CL from a near-term fetus as well as in CL from juvenile animals. In pregnant animals, luteal progesterone probably increased with the duration of gestation.

The values of 20β-hydroxyprogesterone were higher in juvenile giraffes than in the fetus or during early pregnancy. No 20β-hydroxyprogesterone was detectable in a CL from late pregnancy or a CL from a non-pregnant giraffe. The values tended to be inversely related to the progesterone levels.

Detectable levels of 17α-hydroxyprogesterone were present in the fetal ovary, juvenile CL and in one pregnant animal with an early preimplantation blastocyst. In the later stages of gestation, the hormone levels were too low to be detected by TLC.

Thus, it seems that the CL present in the ovaries of fetal and juvenile giraffes are endocrinologically active and similar in function to the CL of adult female giraffes.

INTRODUCTION

In several species, there is tremendous enlargement of the fetal ovaries during gestation. The histological appearance of such gonads has always suggested a secretory function. Amoroso & Rowlands (1951) described the presence of 'interstitial cells' in the medulla of fetal equine ovaries. Previously, Cole, Hart, Lyons & Catchpole (1933) had shown that hypertrophy of the equine fetal ovary was associated with an increase in the urinary oestrogens of the mare.

In yet other species, of which the giraffe is one, there is not only ovarian hypertrophy *in utero* but also the development of CL (Kellas, van Lennep & Amoroso, 1958). The extent to which fetal gonadal hypertrophy and CL formation can be attributed to maternal gonadotrophins or to those of the fetus itself has remained problematical when ovarian luteinization does not persist after birth. The finding of urinary gonadotrophins in pregnant giraffe (Wilkinson & de Fremery, 1940) led Kellas *et al.* (1958) to postulate that these gonadotrophins stimulated the development of the fetal CL. In the giraffe, Kayanja & Blankenship (1973)

have shown that CL are found not only *in utero* but also in all stages of postnatal life up to puberty. The steroidogenic activity of such juvenile giraffe CL, if present, could not be attributed to any residual maternal gonadotrophins.

In this study, luteal progestins from fetal, juvenile and adult giraffes were measured to determine their levels and the differences, if any, in the three developmental categories.

MATERIALS AND METHODS

Reagents

Solvents, when not 'Analar grade', were redistilled immediately before use. All glassware was washed in acid and thoroughly rinsed in deionized water. Thin-layer chromatography (TLC) was carried out on factory-prepared, 20 × 20 cm, 0·25-mm thick silica gel glass plates (Silica Gel F254, Merck, Germany). The [^{14}C]progesterone was purchased from the Radiochemical Centre, Amersham, England. Reagents for gas-liquid chromatography (GLC) and liquid scintillation were purchased from Packard Instruments (Israel) Ltd. Purified gases for GLC were purchased from the East African Oxygen Co.

Tissues

The tissues were obtained from eighteen giraffes of various ages. Two ovaries were obtained from one mid-gestation and one near-term fetus. A fibrotic CL was obtained from another near-term fetus. Six CL from juvenile giraffes of different ages, and those from six animals in early pregnancy were at various stages of development. Two near-term animals yielded large, soft and well-developed CL, and one post-partum giraffe, with incomplete uterine involution, had a corpus albicans.

Methods

Ovaries and CL were obtained within 15 min of shooting and were preserved either in ethanol (two ovaries and fourteen CL) or by freezing on dry ice (four CL). They were subsequently stored deep frozen until required.

Portions were homogenized and extracted twice by reflux with 50-ml aliquots of ethanol. Trace amounts of radioactive progesterone were added for recovery purposes. The supernatants were flash-evaporated to dryness and redissolved in 50-ml aliquots of diethyl ether. The ethereal solutions were washed six times with 10-ml aliquots of deionized water containing three drops of concentrated ammonium hydroxide and were then dried *in vacuo*. The residues were dissolved in 1-ml aliquots of chloroform and stored at 4°C until required.

Aliquots (200 μl) of the residues in chloroform were purified twice by two-dimensional TLC on silica gel F254. Each plate was developed in the first dimension in hexane/methylacetate (5 : 2) twice, then in the second dimension in benzene/methylacetate (2 : 1).

The progestin spots were visualized by short wavelength u.v. light and recovered from the silica gel by overnight elution at 4°C with diethyl ether/chloroform mixtures (1 : 1). The eluates were dried under a stream of nitrogen and redissolved in 200-μl aliquots of carbon disulphide.

Table 1. Progestin values in giraffe

Animal no.	*Reproductive state*	*Wt of animal (kg)*	*μg steroid/g CL (wet weight)*			
			Tissue extracted	*Progesterone*	20β-(*ol*)	17α-(*ol*)
340	Fetus	33	Ovary	9·47	6·38	NM
153	Fetus	52	Ovary	9·41	4·38	1·30
345	Fetus (near term)*	46	Fibrotic CL	1·54	NM	NM
161	Immature	328	CL	ND	17·76	6·12
120	Immature	351	CL	ND	ND	ND
184	Immature	388	CL	7·68	18·06	NM
119	Immature	418	CL	22·60	13·58	14·45
52	Adolescent	500	CL	ND	34·47	6·05
190	Adolescent	510	CL	ND	ND	ND
44	Pregnant (PIB)	613	CL	6·44	2·24	7·15
160	Pregnant (early)	646	CL	6·97	3·05	0·45
117	Pregnant (PIB)	625	CL	11·13	4·08	ND
5/9/72	Pregnant (PIB)*	—	CL	13·40	NM	NM
1	Pregnant (PIB)*	—	CL	33·90	NM	NM
118	Pregnant (early)	624	CL	Lost	ND	ND
153	Pregnant (late)	592	CL	Lost	ND	ND
2	Pregnant (near term)*	—	CL	146·1	NM	NM
172	*Post partum*	668	CA	ND	ND	ND

ND = not detectable; NM = not measured; CL = corpus luteum; PIB = preimplantation blastocyst; 20β-(ol) = 20β-hydroxypregn-4-en-3-one; 17α-(ol) = 17α-hydroxypregn-4-en-3,20-dione.

* Assayed by competitive protein binding.

Tissues from most animals (fourteen) were analysed by GLC on a dual flame ionization chromatograph (Pye-Unicam, Model 24 Series 104) using 5-ft columns of silicone gum (3% SE or 3% OV1) on Diatomite C'Q' (100 to 120 mesh, Pye Unicam). The oven and detector temperatures were set at 250°C whilst the injection port was at 350°C. Gas flow rates were 350, 50 and 35 ml/min for air, nitrogen and hydrogen respectively.

Aliquots (1 to 10 μl) of the standard or the test progestin solutions in carbon disulphide were run through the columns. Minimum sensitivities of 2 to 4 ng were obtained for the various progestins. Unknown concentrations were read from the standard curves. The tissue values were calculated and adjusted for 100% recovery.

In four of the animals, progesterone values were measured by the competitive protein-binding assay of Murphy as previously described by Kayanja, Gombe & Rumney (1974).

RESULTS

Progestin values of individual animals are given in Table 1.

The fetal ovaries contained large amounts (mean$\pm$S.E., 9·44$\pm$0·02 μg/g) of progesterone which were similar to those of the CL in early pregnancy (14·30 $\pm$0·02 μg/g). A fibrotic CL obtained from a near-term fetus contained considerably less progesterone. Four of the six juvenile giraffes had no detectable progesterone in their CL but two others had quite high values. The mean progesterone concentration in the CL of early pregnancy was high but the standard error was large. The near-term CL had a very high progesterone concentration but no progesterone was found in the corpus albicans from the post-partum female.

The values of 20β-hydroxyprogesterone tended to be inversely related to progesterone concentrations. High values were obtained in fetal ovaries (5·38$\pm$1·00 μg/g) and in the CL of four out of six juvenile giraffes (20·97$\pm$4·64 μg/g). The CL of three of the five early pregnant giraffes contained 3·12$\pm$0·53 μg 20β-hydroxyprogesterone/g but none was found in late pregnancy CL or in a post-partum corpus albicans.

The levels of 17α-hydroxyprogesterone were lower than those of the other two progestins. Considerable amounts were found in three out of five juvenile CL (8·87$\pm$2·79 μg/g) and in two out of five early pregnancy CL (3·82$\pm$1·9 μg/g). No 17α-hydroxyprogesterone was found in late pregnancy CL or in a post-partum corpus albicans.

DISCUSSION

Unlike the horse, zebra and human being in which luteinization of fetal and neonatal ovaries has been shown, giraffe develop small multiple CL in fetal and neonatal ovaries and maintain them cyclically until puberty is reached (Kayanja & Blankenship, 1973). Histological examination of the equine fetal ovary (Amoroso & Rowlands, 1951), human neonatal ovary (Govan & Mukherjee, 1950), zebra fetal ovary (Kayanja & Gombe, 1974) and the CL in fetal, neonatal

and juvenile giraffe (Kayanja & Blankenship, 1973) has shown that there is remarkable similarity to the adult CL. This led to the belief that these structures are steroidogenic, and are probably under the influence of maternal gonadotrophins.

McArthur, Short & O'Donnel (1967) showed that testicular incubates from a 9-month-old horse fetus were capable of synthesizing androgens, including testosterone. Kayanja & Gombe (1974) extracted large quantities of progesterone and androstenedione from fetal ovary and testis, respectively, of the zebra at mid-gestation. In this study, we have shown that progestins are found in the ovary of the fetal and CL of the adult giraffe and also in the CL of juvenile giraffes. The occurrence of progestins in the juvenile animals precludes any 'remnant' maternal gonadotrophins being the cause of the CL development. Yet if the offspring's own gonadotrophins are involved in the CL development, it is not clear why there should be a change from the small, multiple CL of fetal, neonatal and juvenile animals to the single, large CL at puberty. It is possible the higher levels of gonadotrophins at puberty might be the basis of the change. The mechanism of preferential development of one Graafian follicle during an oestrous cycle might be analogous, but this mechanism is as yet not understood.

Kayanja & Blankenship (1973) observed that the preimplantation CL in the giraffe did not have the elaborate smooth endoplasmic reticulum found in the later stages of gestation. The association of smooth endoplasmic reticulum with steroidogenic cells was noted by Christiansen & Gillim (1969). Kayanja, Gombe & Rumney (1974) found a correlation between smooth endoplasmic reticulum complexity and progesterone content in the CL of several East African ungulates. It will be seen that the average progesterone content of the CL before implantation and during early pregnancy was only $\frac{1}{10}$ the value of a near-term CL which would agree with the increasing complexity of the smooth endoplasmic reticulum with the duration of gestation that Kayanja & Blankenship (1973) noted. The wide variation of progesterone values in animals with preimplantation blastocysts may also be a reflection of the increasing complexity of smooth endoplasmic reticulum, and hence progestin content, with the duration of gestation.

REFERENCES

Amoroso, E. C. & Rowlands, I. W. (1951) Hormonal effects in the pregnant mare and foetal foal. *J. Endocr.* **7**, 1.

Christiansen, A. K. & Gillim, S. W. (1969) The correlation of fine structure and function in steroid secreting cells with emphasis on those of the gonads. In *The Gonads*, pp. 415–488. Ed. K. W. McKerns. Appleton-Century-Crofts, New York.

Cole, H. H., Hart, G. H., Lyons, W. R. & Catchpole, H. R. (1933) The development and hormonal content of fetal horse gonads. *Anat. Rec.* **56**, 375.

Govan, A. D. T. & Mukherjee, C. C. (1950) Maternal toxaemia and foetal ovarian activity. *J. Obstet. Gynaec. Br. Emp.* **57**, 525.

Kayanja, F. I. B. & Blankenship, L. H. (1973) The ovary of the giraffe (*Giraffa camelopardalis*). *J. Reprod. Fert.* **34**, 305.

Kayanja, F. I. B. & Gombe, S. (1974) Preliminary observations on gonadal ultrastructure and steroids in foetal zebra. *Zentbl. VetMed.* (In press).

Kayanja, F. I. B., Gombe, S. & Rumney, G. (1974) Relation between fine structure and junction of the corpus luteum of wild ruminants. *Zentbl. VetMed.* **12**, 45.

KELLAS, L. M., VAN LENNEP, E. W. & AMOROSO, E. C. (1958) Ovaries of some foetal and prepubertal giraffes. *Nature, Lond.* **181,** 487.

MCARTHUR, E., SHORT, R. V. & O'DONNEL, V. J. (1967) Formation of steroids by equine foetal testis. *J. Endocr.* **38,** 331.

WILKINSON, J. F. & DE FREMERY, P. (1940) Gonadotropic hormones in the urine of the giraffe. *Nature, Lond.* **146,** 491.