

HORMONE LEVELS IN PERIPHERAL PLASMA OF THE AFRIKANER COW

1. PROGESTERONE AND LUTEINIZING HORMONE LEVELS DURING THE OESTROUS CYCLE

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OPSOMMING: HORMOONPEILE IN PERIFERE BLOEDPLASMA VAN AFRIKANERKOEIE.

1. PROGESTEROON EN LUTEINISERENDE HORMOONPEILE GEDURENDE DIE ESTRUSSIKLUS

Progesteron en LH-konsentrasie is in perifere bloedplasma van 3 nie-lakterende Afrikanerkoeie gedurende die estrussiklus bepaal. Bloedmonsters is daaglik versamel vanaf Dag 3 tot Dag 16 van die siklus en daarna elke 8 uur tot 2 dae na estrus. Beide hormone is volgens radioimmunologiese tegnieke bepaal. Progesteroonkonsentrasie het geleidelik toegeneem vanaf Dag 3 tot 'n maksimum waarde van 6,3 - 13,3 ng/ml op Dag 16-17. 'n Tydelike afname in progesteroonkonsentrasie is waargeneem tussen Dag 11 en 14 van die siklus. Binne 48 uur nadat piekwaardes bereik is, het die plasma progesteroonkonsentrasie gedaal tot minder as 1 ng/ml en estrus is gemiddeld 50,7 uur later waargeneem. Alhoewel progesteroonkonsentrasie deurgaans die laagste was tydens estrus (< 1 ng/ml) is 'n klein kortstondige piek kort hierna waargeneem. Herdie piek het 18-25 uur na die preovulêre LH-piek voorgekom en mag moontlik saamval met tyd van ovulasie. Basale plasma-LH-konsentrasies het tussen individue gewissel van 3,9 tot 8,1 ng/ml. 'n Styging vanaf hierdie basislyn is waargeneem sodra die progesteroonkonsentrasie laer as 1 ng/ml gedaal het. 'n Skerp en prominente LH-piek (27,5-45,0 ng/ml) wat waarskynlik korter as 8 uur geduur het, is gedurende die tweede helfte van estrus waargeneem.

SUMMARY:

Concentrations of progesterone and luteinizing hormone were determined in peripheral blood plasma during the oestrus cycle of three non-lactating Afrikaner cows. Blood samples were drawn daily during the luteal phase (Day 3-16) and every 8 hours from Day 17 to 2 days after oestrus. Progesterone was measured by radioimmunoassay and LH by a double antibody radioimmunoassay. Progesterone levels increased gradually from Day 3, reaching maximum values of 6,3-13,3 ng/ml on Day 16-17. A temporary decrease in the progesterone concentration was found between Day 11 and 14 of the cycle. Progesterone levels dropped from peak values to less than 1 ng/ml within 48 hours and were followed by oestrus 50,7 hours later. Progesterone concentrations were lowest at oestrus (< 1 ng/ml) but a small, short lasting peak was observed shortly thereafter. As this peak occurred 18-25 hours after the pre-ovulatory LH surge it might coincide with time of ovulation. Base line LH levels averaged between 3,9 and 8,1 ng/ml for different cows. LH concentration increased as soon as the progesterone level dropped to less than 1 ng/ml. Peak LH levels (27,5-45,0 ng/ml) observed during the latter half of oestrus, were detected at a single sampling only and the release appeared to last less than 8 hours.

Introduction

Beef production in the extensive areas of South Africa is based largely on Afrikaner and/or Afrikaner-type cattle. In comparison with *Bos taurus* breeds the reproductive performance of these Zebu type cattle is often unsatisfactory (Harwin, Lamb & Bisschop, 1967; Bonsma & Skinner, 1969). Although considerable information concerning the reproductive performance of the Afrikaner is available from field trials, data on basic reproductive phenomena are very sparse. For this reason and with the specific and highly sensitive radioimmunoassay techniques now available, the present study was conducted to investigate the endocrinological changes in the Afrikaner during different phases of the reproductive cycle.

Procedure

Three mature, non-lactating Afrikaner cows were observed daily from 05h00 to 19h00 for the occurrence of oestrus. Having ascertained a normal cycling pattern,

blood samples were collected daily at 06h00 between Day 2 and 16 (oestrus = Day 0) and every 8 hours from Day 17 until 2 days after the cessation of oestrus. Blood was drawn from the jugular vein into heparinized tubes, centrifuged and the plasma stored at -15°C until assayed. These observations and sampling were performed at the Vaalharts Research Station, Department of Agricultural Technical Services. Plasma progesterone was determined by radioimmunoassay as described by Botha (1976), using an antibody raised against Progesterone-succinate (J.C. Morgenthal, Department of Human and Animal Physiology, University of Stellenbosch) and luteinizing hormone (LH) by the double antibody radioimmunoassay of Niswender, Reichert, Midgley & Nalbandov (1969). Coefficient of variation for each progesterone standard (n = 20 standard curves) at the different levels was as follows:

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0 pg	:	c.v. = 1,24
250 pg	:	c.v. = 1,78
500 pg	:	c.v. = 1,83
750 pg	:	c.v. = 2,06
1 000 pg	:	c.v. = 2,32
1 500 pg	:	c.v. = 2,40
2 000 pg	:	c.v. = 2,40

Determination of known progesterone quantities indicated that the assay procedure was sensitive enough to distinguish significantly ($P < 0,01$) between 0 and 500 pg/ml and between 500 and 1 000 pg/ml.

Results and Discussion

Cycle length during the sampling period varied from 19,3 days to 21,7 days. Progesterone (ng/ml) and LH (ng/NIH. LH/ml) values obtained during the three individual cycles are given in Tables 1 and 2.

Progesterone

Except for a temporary decrease between Days 11 and 14, progesterone concentrations showed a gradual increase from Day 3, reaching maximum levels of 6,3–13,3 ng/ml on Day 16 and 17 of the cycle (Fig. 1). This pattern of progesterone secretion was very similar in all three cows.

Similar individual variations in progesterone levels to those found in this study have been reported by Hansel & Snook (1970) and Christensen, Hopwood & Wiltbank (1974) and may probably be attributed to size of the corpus luteum (Christensen *et al.* 1974).

The progesterone levels exhibited by the Afrikaner are comparable to those established for dairy cattle by Hansel & Snook (1970), Glencross, Munro, Senior & Pope (1973), Dobson, Midmer & Fitzpatrick (1975) and Lamming & Bulman (1976) but are slightly higher than those reported by Sprague, Hopwood, Niswender & Wiltbank (1971) and Christensen *et al.* (1974) for beef cattle. Peak values were found on Day 16 and 17 of the cycle, this being 4,2 to 5,4 days before the commencement of the next oestrous period. Although some workers reported maximum progesterone levels at a slightly

earlier stage than Days 16–17 (Plotka, Erb, Callahan & Gomes, 1967; Donaldson, Bassett & Thornburn, 1970; Henricks, Hill & Johnston, 1972) others have found the peak values to occur consistently between Day 15 and 17 of the cycle (Stabenfeldt, Ewing & MacDonald, 1969; Hansel & Snook, 1970; Henricks & Dickey, 1970; Christensen *et al.* 1974). The sharp decline in progesterone levels which commenced as early as 4 days prior to oestrus (Lamond, Henricks, Hill & Dickey, 1971; Kanchev, Dobson, Ward & Fitzpatrick, 1976) confirms the pattern suggested by Stabenfeldt *et al.* (1969) and others. The maximum progesterone level in blood thus seems to be in agreement with the greatest weight and progesterone content of the corpus luteum found on Days 15 and 16 of the bovine oestrous cycle (Mares, Zimbleman & Casida, 1962).

A prominent temporary decrease in the progesterone concentration of all 3 cows was found between Day 11 and 14 of the cycle. This phenomenon was also reported by Dobrowolski, Stupnicka & Domanski (1968) and Henricks *et al.* (1970), while Plotka *et al.* (1967) described a significantly lower value on Day 6 in lactating cows. According to Dobrowolski *et al.* (1968) and Hansel & Snook (1970) these temporary fluctuations in progesterone concentration can be associated with the midcycle development and growth of Graafian follicles.

According to the precipitous drop in progesterone levels found during this study, it appears that the functional corpus luteum regresses rapidly once the luteolytic mechanism comes into effect. Progesterone dropped from peak values to less than 1 ng/ml within 31, 39 and 72 hours in the three cows, respectively. From this point (< 1 ng/ml) to the beginning of oestrus the average time elapse was 50,7 hours. In Hereford and Angus cows Lamond *et al.* (1971) established corresponding periods of 34 and 51 hours respectively.

Of interest is the small but consistent peak in progesterone concentration found shortly after the occurrence of oestrus (Fig. 1). The period around oestrus was generally characterized by low progesterone levels (< 1 ng/ml) but a small, short lasting peak was most evident in two of the three cows observed (Table 1).

Fig. 1. Progesterone and LH levels in blood plasma during the oestrous cycle of the Afrikaner (cow 266)

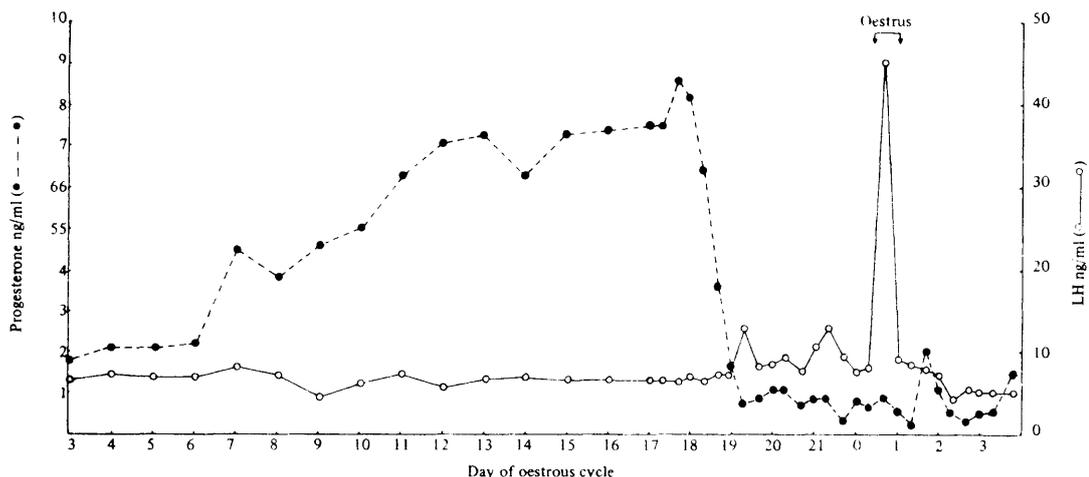


Table 1

Progesterone and LH levels in peripheral blood of Afrikaner cows during the pre- and early post-oestrous stage

Sampling time	Day of cycle	Cow 162		Cow 266		Cow 340	
		Prog ng/ml	LH ng/ml	Prog ng/ml	LH ng/ml	Prog ng/ml	LH ng/ml
6 am	17	4,70	2,30	7,50	7,50	13,30	4,50
2 pm		5,00	4,00	7,50	6,50	12,30	4,75
10 pm		5,90	4,00	8,60	6,25	11,00	4,00
6 am	18	2,30	3,50	8,20	7,00	10,30	4,25
2 pm		1,50	3,25	6,40	6,50	9,60	4,00
10 pm		1,20	1,75	3,60	7,25	5,90	4,50
6 am	19	1,10	5,25	1,70	7,25	4,90	3,75
2 pm		0,75	5,00	0,78	13,00	2,70	5,63
10 pm		1,10	3,25	0,92	8,25	1,50	4,25
6 am	20	–	–	1,10	8,75	0,98	4,00
2 pm		–	–	1,10	9,50	0,53	5,25
10 pm		–	–	0,75	7,75	0,85	4,50
6 am	21	–	–	0,82	10,75	0,47	4,00
2 pm		–	–	0,90	13,00	0,67	6,88
10 pm		–	–	0,35	9,25	0,75	4,25
6 am	0	0,98	4,25	0,84	7,75	0,45	9,50
2 pm		0,85*	37,50*	0,70	8,25	0,24	7,25
10 pm		1,10*	4,75*	0,97*	45,00*	0,48*	9,25*
6 am	1	0,80	4,50	0,59*	9,25*	0,44*	10,50*
2 pm		0,79	5,50	0,25	8,50	0,55*	27,50*
10 pm		0,87	3,00	2,10	8,00	0,44	11,75
6 am	2	0,63	4,25	1,10	7,00	1,40	13,36
2 pm		0,65	4,00	0,57	4,00	0,55	8,25
10 pm		0,74	4,25	0,33	5,75	0,58	11,75

* Samples taken during oestrus

Table 2

Progesterone and LH levels in peripheral blood of Afrikaner cows during the luteal phase

Day of cycle	Cow 162		Cow 266		Cow 340	
	Prog ng/ml	LH ng/ml	Prog ng/ml	LH ng/ml	Prog ng/ml	LH ng/ml
3	1,20	3,50	1,80	6,50	1,20	5,25
4	1,40	4,25	2,10	7,25	0,49	5,50
5	2,10	3,50	2,10	7,00	2,30	8,50
6	2,60	3,00	2,20	7,00	3,30	5,00
7	2,60	4,25	4,50	8,25	4,50	4,75
8	3,50	3,50	3,80	7,25	4,60	4,25
9	3,50	3,50	4,60	4,26	6,10	4,75
10	4,10	4,25	5,00	6,00	7,50	6,00
11	3,20	5,00	6,30	7,25	8,40	5,00
12	4,90	4,00	7,10	5,75	9,30	4,25
13	5,00	5,50	7,30	6,50	8,90	4,25
14	5,10	3,25	6,30	7,00	10,00	2,63
15	6,10	3,00	7,30	6,50	10,10	5,00
16	6,30	5,50	7,40	6,50	13,20	4,00

As this peak occurred approximately 18–25 hours after the pre-ovulatory LH surge, it seems to be closely associated with the time of ovulation. In cycling beef cows Sprague *et al.* (1971) found the average time elapse from LH peak to ovulation to be 29 hours while Christensen *et al.* (1974) reported 24,1 hours. In dairy cows Chenault, Thatcher, Kalra, Abrahams & Wilcox (1975) established a period of 22,25 hours. It was not established whether this peak occurred before or after ovulation. Van Niekerk, Morgenthal & Gerneke (1975) found that there is a small progesterone peak shortly before ovulation in the mare. No similar peak has been found in the cow. The source of progesterone and the possibility that this progesterone peak around the time of ovulation might be peculiar to the Afrikaner (and other Zebu breeds) needs confirmation and further investigation.

Luteinizing Hormone

Base line LH levels averaged between 3,9 ng/ml (cow 162) and 8,1 ng/ml (cow 266). These values were slightly higher than those reported for beef cattle by Sprague *et al.* (1971) and Christensen *et al.* (1974). With the approach of oestrus and the concurrent fall in progesterone levels to below 1 ng/ml, a slight increase in

the plasma LH concentration was noted (Fig. 1). Peak LH levels observed during the latter half of oestrus, were sharply defined and varied from 27,5 to 45,0 ng/ml between individuals. As blood samples were collected at 8 hour intervals, the time and concentration of maximum LH levels could not be accurately determined. According to the work of Schamms & Karg (1969, cited by Henricks *et al.* 1970) elevated LH levels lasted less than 4 to 6 hours. Christensen *et al.* (1974), Chenault *et al.* (1975) and Lemon, Pelletier, Saumande & Signoret (1975) reported elevated LH levels lasting 10–12 hours. As peak LH levels were only found in a single sample during this study, it is concluded that the duration of the LH surge was less than 8 hours.

No luteal phase rise in LH concentration as described by Henricks *et al.* (1970) and Snook *et al.* (1971) was observed during this study.

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References

- BONSMA, J.C. & SKINNER, J.D., 1969. Factors influencing birth date, birth weight and preweaning growth of Afrikaner, Bonsmara and Hereford cattle in the subtropics. *Proc. S. Afr. Soc. Anim. Prod.* 8, 161.
- BOTHA, H.K., 1976. *Die sinkronisasie van estrus asook die involusie van die geslagstelsel van ooie na partus*. Ph.D. proefskrif, Universiteit van Stellenbosch.
- CHENAULT, J.R., THATCHER, W.W., KALRA, P.S., ABRAMS, R.M. & WILCOX, C.J., 1975. Transitory changes in plasma progestins, estradiol and luteinizing hormone approaching ovulation in the bovine. *J. Dairy Sci.* 58, 709.
- CHRISTENSEN, D.S., HOPWOOD, M.L. & WILTBANK, J.N., 1974. Levels of hormones in the serum of cycling beef cows. *J. Anim. Sci.* 38, 577.
- DOBROWOLSKI, W., STUPNICKA, E. & DOMANSKI, E., 1968. Progesterone levels in ovarian venous blood during the oestrus cycle of the cow. *J. Reprod. Fert.* 15, 409.
- DOBSON, H., MIDMER, S.E., & FITZPATRICK, R.J., 1975. Relationships between progesterone concentrations in milk and plasma during the bovine oestrus cycle. *Vet. Rec.* 96, 222.
- DONALDSON, L.E., BASSETT, J.M. & THORBURN, G.D., 1970. Peripheral plasma progesterone concentration of cows during puberty, oestrus cycles, pregnancy and lactation, and the effects of undernutrition or exogenous oxytocin on progesterone concentration. *J. Endocr.* 48, 599.
- GLENCROSS, R.G., MUNRO, I.B., SENIOR, B.E. & POPE, G.S., 1973. Concentrations of oestradiol – 17 β , oestrone and progesterone in jugular venous plasma of cows during the oestrus cycle and early pregnancy. *Acta edocr. (Kbh.)* 73, 374.
- HANSEL, W. & SNOOK, R.B., 1970. Pituitary ovarian relationship in the cow. *J. Dairy Sci.* 53, 945.
- HARWIN, G.O., LAMB, R.D. & BISSCHOP, J.H.R., 1967. Some factors affecting reproductive performance in beef females. *Proc. S. Afr. Soc. Anim. Prod.* 6, 171.
- HENRICKS, D.M., DICKEY, J.R. & NISWENDER, G.D., 1970. Serum luteinizing hormone and plasma progesterone levels during the estrous cycle and early pregnancy in cows. *Biol. Reprod.* 2, 346.
- HENRICKS, D.M., DICKEY, J.F., HILL, J.R. & JOHNSTONE, W.E., 1972. Plasma estrogen and progesterone levels after mating and during late pregnancy and post partum in cows. *Endocrinology*, 90, 1336.
- KANCHEV, L.N., DOBSON, H., WARD, W.R. & FITZPATRICK, R.J., 1976. Concentration of steroids in bovine peripheral plasma during the oestrus cycle and the effect of betamethasone treatment. *J. Reprod. Fert.* 48, 341.
- LAMMING, G.E. & BULMAN, D.C., 1976. The use of milk progesterone radioimmunoassay in the diagnosis and treatment of subfertility in dairy cows. *Br. vet. J.* 132, 507.
- LAMOND, D.R., HENRICKS, D.M., HILL, J.R. & DICKEY, J.F., 1971. Breed differences in plasma progesterone concentrations in the bovine during proestrus. *Biol. Reprod.* 5, 258.

- LEMON, M., PELLETIER, J., SAUMANDE, J. & SIGNORET, J.P., 1975. Peripheral plasma concentrations of progesterone, oestradiol – 17 β and luteinizing hormone around oestrus in the cow. *J. Reprod. Fert.* 42, 137.
- MARES, S.E., ZIMBLEMAN, R.G. & CASIDA, 1962. Variation in the progesterone content in the bovine corpus luteum of the estrual cycle. *J. Anim. Sci.* 21, 266.
- NISWENDER, G.D., REICHERT, L.E., MIDGLEY, A.R. & NALBANDOV, A.V., 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. *Endocrinology*, 84, 1166.
- PLOTKA, E.D., ERB, R.E., CALLAHAN, C.J. & GOMES, W.R., 1967. Levels of progesterone in peripheral blood plasma during the estrous cycle of the bovine. *J. Dairy Sci.* 50, 1158.
- SPRAGUE, E.A., HOPWOOD, M.L., NISWENDER, G.D. & WILTBANK, J.N., 1971. Progesterone and luteinizing hormone levels in peripheral blood of cycling beef cows. *J. Anim. Sci.* 33, 99.
- STABENFELDT, G.H., EWING, L.L. & McDONALD, L.E., 1969. Peripheral plasma progesterone levels during the bovine oestrus cycle. *J. Reprod. Fert.* 19, 433.
- VAN NIEKERK, C.H., MORGENTHAL, J.C. & GERNEKE, W.H., 1975. Relationship between the morphology of and progesterone production by the corpus luteum of the mare. *J. Reprod. Fert. Suppl.* 23, 171.