Effect of high levels of dietary molybdenum and sulphate on SA Mutton Merino sheep. II. Certain aspects of the oestrous cycle and pregnancy

F.E. van Niekerk* and C.H. van Niekerk

Department of Human and Animal Physiology, University of Stellenbosch, Stellenbosch 7600, Republic of South Africa

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Forty-five SA Mutton Merino ewes, 2 to 5 years of age, were divided into three groups. Although all ewes received the same basic diet, the diet of one group (group M) was supplemented with molybdenum and that of another group (MS) with molybdenum and sulphate to induce a secondary copper deficiency. The third group (C) served as a control, and received the basic diet supplemented with copper. At the onset of the breeding season (October), oestrus was suppressed by the induced copper deficiency in groups M and MS. Progesterone concentrations in groups M and MS were lower ($P \leq 0,05$) than that in group C during the oestrous cycle and late pregnancy. Concentration of plasma cholesterol was not affected by the copper deficiency. Concentrations of total plasma copper in both groups M and MS declined during pregnancy whereas that of group C remained constant. Lambs born from ewes in groups M and MS were found to have low concentrations of copper in the plasma (12 μg Cu/dl) and liver (2—20 μg Cu/g DM), which resulted in poor growth and a high pre-weaning mortality rate.

Vyf-en-veertig SA Vleismerino-ooie, van 2 tot 5 jaar oud, is in drie groepe verdeel. Die drie groepe het dieselfde basiese rantsoen ontvang, maar een groep (groep M) het addisionele molibdeen ontvang en 'n ander groep (MS) het addisionele molibdeen en sulfaat ontvang, om sodoende 'n sekondêre kopertekort te induseer. Die derde groep (C) het as kontrole gedien en het slegs die basiese rantsoen met aanvullende koper ontvang. Die voorkoms van estrus by ooie in die kopertekortgroepe (M en MS) is vroeg in die teelseisoen (Oktober) ernstig onderdruk. Progesteroonproduksie by ooie in die kopertekortgroepe (M en MS) is tydens die estrussiklus en gedurende laat dragtigheid onderdruk. Plasmacholesterolkonsentrasies is nie deur die kopertekort beïnvloed nie. Die totale plasmakoperkonsentrasies van ooie in groepe M en MS het afgeneem tydens dragtigheid, terwyl dié van ooie in groep C konstant gebly het. Lammers van ooie uit groepe M en MS het lae plasmakoperkonsentrasies (12 μg Cu/dl) en lae lewerkoperkonsentrasies (2—20 μg Cu/g DM) gehad wat tot swak groei en 'n hoë persentasie voorspeense vrektes gelei het.

Keywords: Copper deficiency, ewes, molybdenum, oestrus, progesterone, sulphate.

* To whom correspondence should be addressed at present address: Elsenburg Agricultural Centre, Private Bag, Elsenburg 7607, Republic of South Africa.

Introduction

Several researchers have suggested that ruminants, suffering from a copper deficiency before or during pregnancy, exhibit impaired reproductive performances. For example, ewes and goats which were known to have aborted (Howell, 1968; Unanian & Feliciano-Silva, 1984), were shown to be suffering from a copper deficiency during pregnancy, whereas low conception rates in dairy cows (Allcroft & Parker, 1949) and a high incidence of anoestrus and suboestrus conditions in cows, and especially in heifers (Munro, 1957), have also been linked to possible copper deficiencies. In addition, Mahadevan & Zubairy (1969) reported that cows, suffering from a copper deficiency, only showed the first signs of oestrus 268 days *post partum*, in contrast to cows which received copper and came in oestrus about 70 days after parturition.

In the female, delayed oestrus, failure to conceive and abortions might be indicative of malfunction in the production of sex hormones. By using ferrets (Donovan & Gledhill, 1981) and rabbits (Suzuki & Baily, 1964), copper was implicated in FSH and LH production as well as the process of ovulation (Hiroi, Sugita & Suzuki, 1965). Von Studnitz & Berezin (1958) found a rise in the concentration of plasma copper in adult women after estrogen supplementation. Ewes suffering from an induced copper deficiency showed a rise in plasma copper during the luteal phase of the oestrous cycle. This rise in plasma copper corresponded with the rise in progesterone concentrations (Van Niekerk, Van Niekerk & Morgenthal, 1988). Copper is also known to act as a catalyst in the production of prostaglandin $F_{2\alpha}$ (Maddox, 1973).

Suttle & Jones (1984) found an increased growth rate in hypocupraemic lambs after supplementing their diet with copper. Woolliams, Woolliams & Wiener (1984) found a high pre-weaning as well as post-weaning mortality amongst hypocupraemic lambs caused by low copper intake.

These findings indicate that a copper deficiency possibly suppresses reproduction in ruminants. However, the precise mechanisms affected are not known. The present study was conducted to determine the influence of an induced copper deficiency on oestrus activity, the oestrous cycle, pregnancy and progesterone production during the oestrous cycle and pregnancy in ewes.

Material and Methods

Phase 1

Forty-five SA Mutton Merino ewes were divided into three equal groups and treated as described by Van Niekerk & Van Niekerk (1989a). Although the groups received the same basic diet, the control group (C) received additional copper, group M additional molybdenum and group MS received additional molybdenum and sulphate, as described by Van Niekerk & Van Niekerk (1989a).

Phase 2

During this phase, which lasted from the beginning of October to the middle of November, the ewes of each group were kept in separate cement-floor paddocks of 20 m × 30 m. Ewes were group fed with the three experimental rations (Van Niekerk & Van Niekerk, 1989a) at a rate of 1,2 kg per animal daily. During this period, ewes were teased twice daily with vasectomized rams fitted with marker harnesses to detect the occurrence of oestrus.

Phase 3

This period lasted from mid-November to mid-December and the same ewes were treated as described in Phase 2. In addition, blood samples were taken from nine ewes, selected at random from each treatment group, during the oestrous cycle. The first sample was taken on the day of oestrus (day 0) and thereafter on days 5, 8, 12 and 16 of the oestrous cycle. All the ewes were mated with fertile rams during oestrus, concurrent with blood sampling. Blood samples (10 ml) were taken with 18G stainless steel needles from the jugular vein of each ewe in heparinized vacuum tubes (Vac U Test) and were centrifuged within 1 h after sampling at 3000 r.p.m. The plasma was removed and 3 ml was stored at −20°C until it was assayed for progesterone. The remaining plasma was kept at 4°C and was analysed for cholesterol, high density lipoprotein (HDL) cholesterol, copper and trichloroacetic acid (TCA) soluble copper on the day of sampling.

Phase 4

After mating, blood samples were taken from all the ewes every second week throughout pregnancy. During pregnancy, the ewes were housed and fed as described in Phase 2. During the last six weeks of pregnancy, intake was increased to 2,0 kg per ewe per day. The number of lambs born to each ewe as well as each individual birth mass were recorded. Ewes were housed individually with their lambs in a feeding shed to ensure that the lambs only suckled from their mothers. Blood samples were taken from both ewes and lambs. The first samples were taken within 24 h after birth and every second week thereafter till the lambs were 56 days old. Lambs were weighed at birth, at 36 days and again at 60 days of age. The livers of all the lambs that had died during this period were removed and analysed for trace elements. During lactation, the intake of the ewes in all groups was increased to 1,6 kg of the corresponding experimental diet plus 0,4 kg lucerne. During pregnancy (Phase 4), 20 ml blood samples were taken from each ewe every second week, and 10 ml of each sample was stored at −20°C for selenium determinations. The remaining 10 ml was centrifuged within 3 h after sampling at 3000 r.p.m. and the plasma was stored at −20°C for the determination of copper, zinc and progesterone.

Liver trace elements as well as blood selenium, plasma copper, zinc and TCA-soluble plasma copper were analysed according to methods described by Van Niekerk & Van Niekerk (1989a). Concentrations of plasma unconjugated progesterone were determined by radio-immunoassay as described by Van Niekerk & Morgenthal (1982). Total plasma cholesterol as well as HDL-cholesterol concentrations were determined spectrophotometrically using standard kits (Clinical Sciences Diagnostics, Catalogue no. H1060 and Clinical Sciences Diagnostics, Catalogue no. C1065, respectively).

Results were evaluated statistically according to a one-way variance and covariance analysis, using the standard BMDP program P1V (Dixon, 1982). In cases where only mean values are presented, the standard deviation (± *SD*) of the mean is also indicated.

Results

The period July to September, during which the copper deficiency was induced (Van Niekerk & Van Niekerk, 1989a), is generally accepted as the time of year when sexual activity of SA Mutton Merino sheep is at its lowest. Therefore, it may be postulated that ewes in groups M and MS suffered from a severe copper deficiency at the commencement of the breeding season in October.

The results presented in Table 1 indicate that 82% of the ewes in group C came into oestrus during the six-week period from the beginning of October to middle November. During the same period only 72% and 54% of the ewes in groups M and MS, respectively, exhibited oestrus. By the first week in December, all the ewes of the three treatment groups had come into oestrus. At that time, the ewes were constantly in direct contact with the teaser rams for nearly 70 days.

Table 1 Percentage ewes in oestrus during the first six weeks of the experimental period (October to mid-November; Phase 2)

	% Ewes in oestrus during experimental period (weeks)					
Group	1	2	3	4	5	6
C	27	63	72	82	82	82
M	18	27	27	63	63	72
MS	0	0	27	36	36	54

Concentrations of plasma progesterone, total plasma copper and TCA-soluble plasma copper recorded on days 1, 5, 8, 12 and 16 of the oestrous cycle are presented in Table 2. Concentrations of plasma progesterone on these days illustrate the activity and development of the corpus luteum at the different stages of the oestrous cycle.

The mean concentrations of plasma cholesterol did not differ significantly between treatment groups at the same sampling, but were lower ($P \leq 0,05$) at day 12 of the oestrous cycle within the same group, as indicated in Table 3. Concentrations of high density lipoprotein (HDL) cholesterol were not affected by the copper deficiency.

About 54%, 45% and 72% of the ewes lambed in groups C, M and MS, respectively. Results reported in Phase 4 include only those of ewes that lambed.

Table 2 Mean (± *SD*) concentrations of plasma progesterone, totat plasma copper and TCA-soluble plasma copper of the ewes during the oestrous cycle (Phase 3)

Parameter	Group[a]	Day of oestrous cycle 1	5	8	12	16
Progesterone (ng/ml)	C	0,42 ± 0,13	0,66 ± 0,15	1,07 ± 0,44	1,63 ± 0,28	0,95 ± 0,38
	M	0,32 ± 0,13	0,54 ± 0,18	0,81 ± 0,26	1,36 ± 0,23	1,26 ± 0,43
	MS	0,35 ± 0,08	0,54 ± 0,26	0,91 ± 0,44	1,28 ± 0,35	0,78 ± 0,35
Plasma copper (μg/dl)	C	115 ± 11	123 ± 14	117 ± 25	124 ± 12	131 ± 16
	M	132 ± 34	159 ± 24	148 ± 25	169 ± 19	164 ± 29
	MS	102 ± 23	128 ± 27	115 ± 20	146 ± 30	133 ± 23
TCA-soluble copper (μg/dl)	C	107 ± 8	115 ± 9	107 ± 25	114 ± 10	122 ± 8
	M	93 ± 9	93 ± 10	81 ± 7	94 ± 10	105 ± 18
	MS	80 ± 10	80 ± 15	84 ± 18	85 ± 7	77 ± 11

[a] Samples were taken from nine ewes per group.

Table 3 Mean (± *SD*) concentrations of plasma cholesterol and HDL cholesterol in the ewes during the oestrous cycle (Phase 3)

Component	Group*	Day of oestrous cycle 1	12
Cholesterol (mmol/l)	C	1,15[a] ± 0,46	0,64[b] ± 0,21
	M	1,31[a] ± 0,22	0,78[b] ± 0,14
	MS	1,09[a] ± 0,28	0,77[b] ± 0,27
HDL cholesterol (mmol/l)	C	0,65[a] ± 0,29	0,40[b] ± 0,19
	M	0,79[a] ± 0,13	0,30[b] ± 0,10
	MS	0,56[a] ± 0,22	0,38[b] ± 0,21

* Samples were taken from nine ewes per group.

[a,b] Values in the same row with different headings differ significantly ($P \leq 0,05$).

Up to mid-pregnancy, treatment had no significant effect on the concentrations of plasma progesterone in ewes of all three groups (Table 4). From week 12 of pregnancy, the mean progesterone concentrations of ewes in group MS were significantly lower ($P \leq 0,05$) than the concentrations for ewes in groups C and M. From week 18 of pregnancy, the mean progesterone concentration of ewes in group M was also lower ($P \leq 0,05$) than that of ewes in the control group.

Total concentrations of plasma copper (Table 5) of ewes in groups M and MS were higher ($P \leq 0,05$) than those of the ewes in the control group during the first four weeks of pregnancy. The total concentrations of plasma copper in the ewes of group C increased from 86 μg/dl in the second week of pregnancy to 110 μg/dl in week 20. During the same period, the concentrations of plasma copper in ewes of group M declined from 130 μg/dl to 108 μg/dl and in group MS from 105 μg/dl to 81 μg/dl. This decrease in plasma copper in group MS resulted in significantly lower values ($P \leq 0,05$) compared to groups C and M after week 16 of pregnancy.

Table 4 Mean concentrations (ng/ml) of plasma progesterone in the ewes during pregnancy (Phase 4)

Weeks pregnant	Plasma progesterone concentration Group C	Group M	Group MS
2	3,79[a]	3,40[a]	3,70[a]
4	4,37[a]	4,25[a]	3,71[a]
6	3,95[a]	3,80[a]	4,08[a]
8	5,04[a]	3,70[a]	4,96[a]
10	6,87[a]	5,95[a]	6,06[a]
12	9,08[a]	8,75[a]	6,18[b]
14	12,20[a]	9,75[ab]	8,03[b]
16	12,61[a]	9,20[a]	11,06[a]
18	16,00[a]	10,86[b]	13,25[ab]
20	19,50[a]	14,65[b]	13,56[b]

[a,b] Values in the same row with different headings differ significantly ($P \leq 0,05$).

Table 5 Mean concentrations (μg/dl) of total plasma copper in the ewes during pregnancy (Phase 4)

Weeks pregnant	Plasma copper concentration Group C	Group M	Group MS
2	86[a]	130[b]	105[c]
4	88[a]	140[b]	98[c]
6	96[a]	142[b]	97[a]
8	106[a]	122[a]	106[a]
10	123[a]	136[a]	125[a]
12	113[a]	138[b]	121[ab]
14	80[a]	118[b]	76[a]
16	111[a]	106[a]	95[a]
18	120[a]	118[a]	90[b]
20	110[a]	108[a]	81[b]

[a,b,c] Values in the same row with different headings differ significantly ($P \leq 0,05$).

In group C, the concentration of plasma zinc (Table 6) remained constant throughout pregnancy. In contrast, ewes in groups M and MS showed a rise in the concentrations of plasma zinc after week 6 of pregnancy. Although the mean concentration of plasma zinc in group M was consistently higher than that in group C after week 6 of pregnancy, it was not statistically significant. The increase in concentration of plasma zinc in group MS was more marked and the concentrations were consistently higher ($P \leq 0,05$) than that of group C after week 8 of pregnancy. The rise in concentration of plasma zinc (Table 6) coincided with the decrease in total plasma copper concentrations (Table 5) in groups M and MS.

Ewes in the control group showed a mild decrease in the concentrations of blood selenium (Table 7) during pregnancy, but the concentration still remained within normal limits. A decrease in concentrations of blood selenium in groups M and MS was also noted during pregnancy. The mean values of group MS were consistently lower ($P \leq 0,05$) than those of groups C and M throughout pregnancy. The selenium concentrations in group MS were, however, still above the minimum concentrations and were not indicative of a deficiency.

Lambs in groups M and MS were found to have extremely low concentrations of plasma copper (*ca.* 11 µg/dl) at birth (Table 8). These low concentrations were maintained up to 56 days of age, when blood sampling was discontinued. Lambs in group C had a relatively low concentration of plasma copper (30 µg/dl) at birth, which increased to 63 µg/dl during the second week, and it remained at this level for the rest of the experimental period.

Table 6 Mean concentrations (µg/dl) of plasma zinc in the ewes during pregnancy (Phase 4)

Weeks pregnant	Plasma zinc concentration		
	Group C	Group M	Group MS
2	70[a]	64[a]	60[a]
4	58[a]	64[a]	61[a]
6	71[a]	82[a]	95[a]
8	68[a]	78[a]	80[a]
10	70[a]	82[ab]	86[b]
12	71[a]	82[ab]	92[b]
14	68[a]	80[ab]	91[b]
16	80[a]	98[ab]	110[b]
18	68[a]	80[a]	92[b]
20	65[a]	84[b]	92[b]

[a,b] Values in the same row with different headings differ significantly ($P \leq 0,05$).

Table 7 Mean concentrations (ng/ml) of blood selenium in ewes during pregnancy (Phase 4)

Weeks pregnant	Blood selenium concentration		
	Group C	Group M	Group MS
2	160[a]	153[a]	115[b]
4	149[a]	136[a]	98[b]
6	141[a]	130[a]	89[b]
8	122[a]	108[ab]	84[b]
10	123[a]	108[ab]	80[b]
12	120[a]	109[ab]	83[b]
14	110[a]	116[a]	92[a]
16	132[a]	124[a]	90[b]
18	128[a]	119[a]	78[b]
20	120[a]	106[a]	67[b]

[a,b] Values in the same row with different headings differ significantly ($P \leq 0,05$).

Table 8 Mean (± *SD*) concentrations of blood selenium, plasma copper and zinc in the ewes and their lambs from birth to 56 days of age (Phase 4)

Mineral	Group	Ewes or lambs	Time (weeks) after birth of lambs			
			0	2	4	6
Selenium (ng/ml)	C	Ewes	86 ± 10	84 ± 12	87 ± 19	71 ± 15
		Lambs	77 ± 12	74 ± 9	74 ± 10	67 ± 12
	M	Ewes	74 ± 15	70 ± 17	68 ± 11	55 ± 15
		Lambs	75 ± 11	69 ± 12	66 ± 5	56 ± 10
	MS	Ewes	60 ± 9	57 ± 7	62 ± 3	50 ± 2
		Lambs	71 ± 17	64 ± 16	66 ± 21	72 ± 9
Plasma copper (µg/dl)	C	Ewes	100 ± 7	108 ± 17	146 ± 17	74 ± 6
		Lambs	30 ± 6	63 ± 26	62 ± 15	62 ± 12
	M	Ewes	98 ± 14	101 ± 10	111 ± 18	94 ± 16
		Lambs	13 ± 5	12 ± 4	11 ± 1	12 ± 4
	MS	Ewes	71 ± 13	80 ± 14	96 ± 11	94 ± 3
		Lambs	11 ± 1	11 ± 1	11 ± 2	13 ± 6
Zinc (µg/dl)	C	Ewes	71 ± 9	64 ± 8	70 ± 5	66 ± 10
		Lambs	98 ± 19	116 ± 24	128 ± 9	88 ± 5
	M	Ewes	77 ± 12	85 ± 9	90 ± 13	88 ± 8
		Lambs	105 ± 25	130 ± 24	113 ± 26	110 ± 28
	MS	Ewes	70 ± 14	75 ± 9	97 ± 20	88 ± 9
		Lambs	130 ± 24	115 ± 18	97 ± 19	97 ± 13

Total concentrations of plasma copper in the ewes of group MS increased from 71 μg/dl to 94 μg/dl during the 56 days after birth of the lambs, whereas those of groups C and M remained constant.

The lambs of all three groups were found to have a higher concentration of plasma zinc than the ewes throughout this six-week period (Table 8). Although the ewes in group MS had lower blood selenium concentrations than the ewes in groups C and M, there were virtually no differences between concentrations of blood selenium of lambs from the three groups. From the mean mass of the surviving lambs (Table 9) it is evident that the growth of lambs in group M, and particularly group MS, was severely suppressed when compared to that of the lambs in group C (Table 9).

Table 9 Mean body mass (kg ± *SD*) of lambs born in the three groups from birth to 56 days of age (Phase 4)

	Mean mass of lambs		
Age	Group C	Group M	Group MS
Birth	4,12 ± 1,18 n = 9	4,18 ± 0,83 n = 7	3,58 ± 1,26 n = 12
32 Days	10,31 ± 4,12 n = 8	11,91 ± 3,69 n = 6	6,47 ± 1,18 n = 4
56 Days	14,50 ± 4,75 n = 7	13,39 ± 5,59 n = 6	8,26 ± 1,02 n = 4

n = Number of surviving lambs in groups at time of weighing.

Lamb mortalities before 56 days of age were recorded to be 23% in group C, 15% in group M and 66% in group MS. The concentrations of liver copper in lambs that died were 90 and 108 μg Cu/g DM for the two lambs in group C, 68 μg Cu/g DM for the one in group M, and varied between 2 and 20 μg Cu/g DM for the eight dead lambs in group MS.

Discussion

By supplementing ewes with molybdenum (group M) and molybdenum plus sulphate (group MS), an effective secondary copper deficiency was induced in these two groups. The mean concentrations of liver copper in ewes of groups M and MS after receiving the diets for a period of three months, were 28 μg Cu/g DM and 26 μg Cu/g DM, respectively (Van Niekerk & Van Niekerk, 1989a). It is generally accepted that concentrations of liver copper of less than 50 μg Cu/g DM are indicative of a deficiency (Underwood, 1977). Concentrations of plasma copper, the biological availability thereof and the formation of biologically inactive thiomolybdate-bound plasma copper during this three-month period have been described by Van Niekerk & Van Niekerk (1989a). Higher than normal molybdenum concentrations in the diet of ruminants lead to the formation of thiomolybdates in the body. Thiomolybdate formation may be increased when the sulphur concentration in the diet is high at the same time (Van Niekerk & Van Niekerk, 1989a). These thiomolybdates bind plasma copper and render it unavailable for biological functions. This biologically inactive thiomolybdate-bound copper may prevail for an indefinite period in the bloodstream, resulting in misleading values for total biologically available plasma copper (Van Niekerk & Van Niekerk, 1989a).

Ewes in groups M and MS, which suffered from a copper deficiency at the commencement of the breeding season, showed prolonged periods of seasonal anoestrus when compared to ewes in the control group (Table 1). This is in agreement with the findings of Munro (1957) and Mahadevan & Zubairy (1969). It is well known that the presence of rams stimulates oestrus in ewes at the beginning of the breeding season (Martin, Oldham, Cognie & Pearce, 1986). During this study, the constant presence of teaser rams for a period of 70 days did not bring the ewes into oestrus at an earlier stage of the breeding season.

Production of progesterone during the oestrous cycle in ewes of groups M and MS was lower when compared to the concentrations of plasma progesterone of ewes in group C (Table 2). Concentrations of progesterone found in group C corresponded with the normal values described by Stabenfelt, Holt & Ewing (1969).

Ewes in group MS had lower ($P \leq 0,05$) concentrations of plasma progesterone throughout the second half of pregnancy when compared to the concentrations in ewes of group C (Table 6). Ewes in group M had lower ($P \leq 0,05$) concentrations of plasma progesterone only during the last month of pregnancy when compared to group C. It therefore seems that progesterone production in group M, and to a greater extent in group MS, was suppressed by the copper deficiency.

During the oestrous cycle, the concentrations of total plasma copper and the TCA-soluble plasma copper never declined below 80 μg/dl in any of the three groups. Under conditions of primary copper deficiency, a concentration of less than 60 μg/dl of plasma copper is regarded as a sign of copper deficiency (Underwood, 1977). It is nevertheless clear that, according to the clinical symptoms of a copper deficiency found in this study (*viz.* anaemia, loss of crimp in the wool, loss in wool production, loss in body mass, the high pre-weaning mortality rate of the lambs and the abnormally low liver copper concentrations of those sheep), the TCA-soluble plasma copper is not a true indication of the biologically available plasma copper as discussed by Van Niekerk & Van Niekerk (1989a). Despite high concentrations of biologically unavailable thiomolybdate-bound circulating plasma copper, the total concentration of plasma copper in groups M and MS declined during pregnancy. This is in agreement with results of Butler (1963), who found a decline in the concentrations of plasma copper during pregnancy in ewes suffering from a copper deficiency. According to Van Niekerk & Van Niekerk (1989a), approximately 65% of the total plasma copper is TCA soluble under conditions of molybdenum- and sulphate-induced copper deficiency. Therefore, if the total plasma

copper concentration of group MS (Table 6) during the second half of pregnancy is taken into consideration, the concentration of TCA-soluble plasma copper would be in the vicinity of 50 μg/dl, which is indeed indicative of a copper deficiency (Underwood, 1977). The concentrations of liver copper in groups M and MS, however, suggested that these ewes suffered from a severe copper deficiency (Van Niekerk & Van Niekerk, 1989a). The present findings are in agreement with those of Van Niekerk, Van Niekerk & Morgenthal (1988), who stated that copper plays an important role in progesterone production in ewes.

The copper deficiency did not appear to affect plasma cholesterol concentrations (Table 3), although Allen & Klevay (1978) found a rise in serum cholesterol in rats suffering from a copper deficiency. The present results are in agreement with those of Sherman (1981), who did not find an increase in serum cholesterol in rats suffering from a copper deficiency. No differences in HDL-cholesterol concentrations between treatment groups were found (Table 3).

The rise in concentration of plasma zinc (Table 6) in copper deficient ewes of groups M and MS, which coincided with the decline in concentration of total plasma copper, is conspicuous. None of the ewes in the three experimental groups appeared to suffer from a selenium deficiency during pregnancy (Table 7) or during the first 56 days of lactation (Table 8), as all concentrations recorded were more than 50 ng/ml, which is regarded as indicative of a deficiency (Underwood, 1977). Lambs born from the copper deficient ewes in groups M and MS had extremely low concentrations of plasma copper (11 μg/dl) at birth, which remained low throughout the experimental period. The liver copper concentrations of the lambs which died in group MS varied between 2 and 20 μg Cu/g DM. This supports the contention that a severe copper deficiency existed in the ewes of group MS as well as in their offspring. The low concentrations of copper in the lambs might have resulted in poor growth as well as a high mortality rate (Table 9). None of the lambs showed signs of enzoötic ataxia while they were kept with the ewes in the feeding shed.

It is concluded that a copper deficiency delays the onset of oestrus after the non-breeding season. The possible suppressive effect of a molybdenum- and sulphate-induced copper deficiency on the production of gonadotrophic hormones in rams has been discussed by Van Niekerk & Van Niekerk (1989b). In a similar study on heifers, Phillippo, Humphries & Atkinson (1987) also found a delay in the onset of oestrus and a suppressive effect on LH production. They stated that high molybdenum intake, and not the low concentration of body copper, was possibly responsible for this effect and based their conclusions on their findings that the low concentrations of plasma copper in iron-supplemented heifers were the same as those of molybdenum-supplemented animals. These authors, however, did not take the phenomenon of thiomolybdate complex formation in blood into consideration. It is therefore possible that, even with the same low concentration of total plasma copper that were found in the molybdenum as well as the iron-induced copper-deficient groups, the molybdenum group suffered from a more severe copper deficiency than the iron group.

The low progesterone production, especially during late pregnancy, of the copper deficient ewes in groups M and MS may cause abortions as described by Howell (1968) and Unanian & Feliciano-Silva (1984). This is especially so when pregnant ewes are subjected to stressful conditions, which, according to Van Niekerk & Morgenthal (1982), may suppress the production of progesterone. This is also in agreement with the findings of Van Niekerk *et al.* (1988) that copper may play an important role in progesterone production.

One of the most outstanding effects of a copper deficiency on reproduction found in the present study is the severe suppression of lamb growth and the concomitant increase in lamb mortality, which is in agreement with the findings of Woolliams *et al.* (1984) and Suttle & Jones (1984).

This study as well as other investigations (Phillippo *et al.*, 1987; Van Niekerk *et al.*, 1988; Van Niekerk & Van Niekerk, 1989a; Woolliams *et al.*, 1984; Howell, 1968) provide supporting evidence that a copper deficiency, resulting from either a low copper intake or from induction by antagonists, influences the reproductive processes in several ways, including delayed onset of oestrus, impaired production of several of the reproductive hormones, male infertility, abortions and poor lamb growth.

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