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THE INFLUENCE OF EXCESS FLUORINE INTAKE IN THE DRINKING WATER ON REPRODUCTIVE EFFICIENCY IN BOVINES

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INTRODUCTION

Fluorine is widely distributed in nature in a combined form with a number of naturally occurring minerals, particularly phosphorus. In certain areas where rock phosphate is found, the fluorine content of the soil may reach 0.15 per cent and of the water, especially from underground sources, 15 parts per million (Garner, 1961).

Investigations carried out in South Africa indicate that in many parts of this country the fluorine content of soil and water far exceeds the levels mentioned by Garner. Steyn (1949) reports that an analysis of thousands of samples of water from various parts of South Africa revealed only a few that were completely free from fluorine, and that in certain areas of the Transvaal it exceeded 60 ppm. Ockerse (1941) who investigated fluorosis in human beings, states that thousands of persons in South Africa obviously suffer from endemic dental fluorosis, and found that fluorine concentration in drinking water varies from 0 to 36.67 ppm.

According to Mitchell & Edman (1952) the hazards to livestock arise largely from the use of fluorine-containing minerals as supplements to farm rations, such as superphosphates. Also from partially defluorinated phosphates obtained from phosphatic rock, drinking water in which the fluorine content attains levels of several ppm, and from pastures in the neighbourhood of factories emitting gases and dustcontaining fluorine.

In the extensive investigations into fluorosis in various countries and in the resultant voluminous literature on the subject, attention is focused mainly on the toxic effects on the skeletal system and on the general health of animals. In the relatively few instances in which reproduction is mentioned, it receives only casual consideration, and many of the reports on this aspect of the problem are contradictory.

Mitchell & Edman (1952) concluded that experimental fluorosis produced by up to 320 ppm of the total feed on a dry basis, did not affect the reproductive processes. Oestrus, impregnation and normal parturition with normal healthy young occurred at all levels of fluorine intake. They admit, however, that there was a decline in the frequency of oestrus as well as delayed oestrus after parturition, but they ascribe this to reduced feed consumption.

Phillips, Hart & Bohstedt (1934) state that the data obtained indicate that no functional failure of the reproductive processes occurred in chronic toxicosis, but the accuracy of this conclusion can be questioned by their subsequent statement that " the occurrence of oestrus following parturition was significantly delayed in the fluorine-fed animals ".

Neeley & Harbaugh (1954) observed that in a herd of 40 dairy cows born and raised in an area having a water supply with an average of 4 to 5 ppm fluorine, milk yield and breeding efficiency were not affected. This is in agreement with the finding of most workers that a concentration of under 5 ppm in water is not toxic.

Hobbs & Merriman (1962) judged on the basis of the number of calves raised in each successive season that fluorine had no effect on reproduction. Yet they report that the number of calves raised was reduced from 100 per cent in the first season to $56 \cdot 2$ per cent in the fifth season, and further that cows in the groups receiving 78 and 108 ppm fluorine in the feed, had lower reproduction records than those in the groups receiving 10 to 50 ppm.

Udall & Keller (1952) investigated fluorosis in cattle in nine herds in the Columbia River Valley and found a high degree of infertility in four of these with delayed onset of oestrus after calving a prominent symptom.

MATERIALS AND METHODS

An opportunity to observe the influence of various levels of fluorine in the drinking water over four breeding seasons on reproduction, was presented by an experiment reported on by Myburgh (1965). This aimed at determining the effect of fluorine and phosphate on the general health of cattle.

Fifty Afrikaner heifers, all from $2\frac{1}{2}$ to $2\frac{3}{4}$ years old and free from tuberculosis, brucellosis and from the various coital diseases, were divided into five groups of ten. They were grazed in a camp without provision for drinking water and were brought into five different paddocks for about two hours every morning. Here each group had free access to drinking water medicated for the specific groups as follows:—

Group 1:5 ppm fluorine.

Group 2: 8 ppm fluorine.

Group 3: 5 ppm fluorine and defluorinated superphosphate.

Group 4: 8 ppm fluorine and defluorinated superphosphate.

Group 5: 12 ppm fluorine and defluorinated superphosphate.

An analysis of the drinking water prior to the commencement of the experiment showed that its fluorine content was negligible, namely under 0.3 ppm.

The defluorinated superphosphate extract given to Groups 3, 4 and 5 was prepared according to the method of Truter & Louw (1952) and supplied 1 gm of phosphorus per gallon of water.

Breeding was started nine months after the commencement of the experiment. During the breeding season (September to May) observations for oestrus were made every morning while the animals were in the paddocks and those displaying evidence of oestrus were served. During the first two seasons they were served naturally while artificial insemination with fertile semen was applied during the last two seasons.

Detailed breeding records were kept in respect of each animal, and where behavioural aberrations were noticed the genitalia were examined by rectal palpation. In this manner genital hypoplasia was detected in one of the heifers in Group 1 and, although she was kept in the experiment throughout its duration, she never conceived. She was, therefore, not considered in the evaluation of the results and for this reason Group 1 had only nine.

RESULTS

The yardstick for determining the reproductive efficiency of a herd is the number of young born within a specified period and the number of services required to produce them.

		Breeding Seasons					
Group		1	2	3	4		
1 9 Heifers 5 ppm fluorine	No. served No. of services	9	8	8 12	6		
	No. conceived Services per conception Calving rate	8 1 · 12 88 %	8 1·00 88%	6 2.00 66.7%	4 2·00 44·4%		
10 Haiferra	Nie comod	10	6	0	0		
8 ppm fluorine	No. of services.	10	6	15	11		
	No. conceived Services per conception Calving rate	8 1·75 80%	6 1.00 60%	6 2 · 50 60 %	5 2·20 50%		
3		10		0	E		
5 ppm fluorine, plus de-	No. of services	10	10	9	6		
fluorinated superphos-	No. conceived	8	9	1.28	2.00		
1	Calving rate	80%	90%	70%	30%		
10 Heifers	No. served	10	6	10	8		
8 ppm fluorine, plus de-	No. of services	15	6	13	11		
phate	Services per conception	1.67	1.00	2.60	11.00		
5	Calving rate	90%	60 %	50%	10%		
10 Heifers	No. served.	9	5	6	7		
12 ppm fluorine, plus	No. of services	10	5	11	15		
phosphate super-	Services per conception	1.11	1.25	$5 \cdot 50$	15.00		
	Calving rate	90%	40 %	20%	10%		
TOTAL for 49 heifers	No. served.	48	34	42	34		
	No. of services	60	35	60	51		
	Services per conception	1.43	1.06	2.31	3.64		
	Calving rate	85.7%	67.3%	53.1%	28.4%		

 TABLE 1.—Breeding efficiency of five groups on different levels of fluorine intake, with and without the addition of defluorinated superphosphate over four breeding seasons

The data contained in the summarized breeding results in Table 1 show that the reproductive performance in the first season was normal in all respects; the 49 heifers produced 42 calves giving a calving rate of 85.7 per cent while the conception rate was 1.43 services per conception. Any slight inter-group difference in fertility level at this stage was in favour of lots 4 and 5, each of which yielded nine calves as against eight in each of the other three. It is noteworthy that Group 5 also had the best conception rate with 1.11 service per conception.

A prominent feature revealed by the records for the second season is that only 34 of the 49 heifers were served, the remaining 15 showing prolonged anoestrus after calving. It is significant that 13 of the latter belonged to Groups 2, 4 and 5 which received the high fluorine levels of 8, 8 and 12 ppm respectively, while only two of the 5 ppm Groups 1 and 3 showed this extended post-calving anoestrus. The conception rate of the 34 animals that did resume ovarian activity before the end of the season was very good, averaging 1.06 services per conception.

In the third season 42 cows were served amounting to an increase of eight over the previous season. This improvement in post-calving resumption of ovarian activity is more apparent than real and must be attributed to the long period of sexual rest enjoyed by the 15 animals not served the previous year. In comparison with the two previous seasons the conception rate was poor, the deterioration being particularly marked in the three groups receiving more than 5 ppm fluorine. The number of services per conception was $2 \cdot 50$, $2 \cdot 60$ and $5 \cdot 50$ respectively as against $2 \cdot 00$ and $1 \cdot 28$ for the two 5 ppm groups. The poor conception rate of the ten cows in Group 5 resulted in only two calves being born to them that season.

The fourth season was characterized by an all-round decline in fertility. Contrary to expectations the percentage of cows that came into oestrus and were served was lower in the 5 ppm group than in the others with the higher fluorine intake. The prolonged anoestrus in eight of the 19 animals is largely due to the high fertility displayed by them in the preceding three seasons when the eight cows concerned displayed normal resumption of the oestrous cycle after each calving and conceived readily. Further it is not improbable that the strain of regular conception and calving up to this point might have been aggravated by the cumulative effect of the 5 ppm intake of fluorine in the drinking water over a four-year period.

As regards the conception rate, however, both the breeding results and the clinical examination of the genitalia reveal evidence of pronounced disturbance in ovulation and fertilization in the three high fluorine intake lots during the fourth season, the number of services per conception having risen to $2 \cdot 20$, $11 \cdot 00$ and $15 \cdot 00$ for Groups 2, 4 and 5 respectively. Thus Group 4 yielded only one calf from 11 services applied to eight cows and Group 5 produced one calf from seven cows after 15 services. Of the 30 cows in these three groups, 23 were served in the fourth season by a total of 37 services from which seven calves were born. This gives a calving rate of $23 \cdot 3$ per cent and a conception rate of $5 \cdot 28$ services per conception. In the two 5 ppm fluorine groups only 11 of the 19 available cows came into oestrus and were bred. They received a total of 14 services which produced seven calves, representing a calving rate of $36 \cdot 8$ per cent and a conception rate of $2 \cdot 00$ services per conception.

Apart from unduly prolonged post-calving anoestrus caused by static ovaries occurring in all the animals from the first calving till the termination of the experiment, clinical examination of the ovaries did not reveal important aberrations during the first two seasons in the 19 heifers that received 5 ppm fluorine. During the <u>third</u> and fourth seasons there was one case of a follicle rupturing prematurely, one with anovulation, one showing a corpus luteum persistence and one cow developed a large ovarian cyst in the fourth year.

The 20 receiving 8 ppm fluorine (Groups 2 and 4), showed rather more evidence of functional ovarian disturbance in the last two breeding seasons when there were seven cases of delayed ovulation, three of anoestrus and four showing corpus luteum persistence.

While the ten animals with a fluorine intake of 12 ppm in the crinking water also did not reveal any noteworthy interference with ovarian activity excepting static ovaries in the first two seasons, quite marked deviations from the normal were observed

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in the third and fourth breeding seasons. These included five cases of anovulatory oestrus, one of delayed ovulation, one with corpus luteum persistence and one showing resorption of the foetus. The relative high incidence of anovulation is important as this was obviously one of the principal causes of the poor conception rate.

An interesting feature of the results is the wide difference in the fertility level appearing in the third and fourth seasons between the two Groups, 2 and 4, which received 8 ppm fluorine. This divergence manifested itself after the two lots had showed a marked similarity in breeding efficiency in the first two seasons. In the third and fourth seasons the ten cows in Group 2 produced 11 calves from 26 services giving a conception rate of 2.36 services per conception and a calving rate of 55 per cent per year. Over the same period the ten cows in Group 4 delivered six calves from 24 services, the conception rate being 4.00 services per conception and the calving rate 30 per cent per annum. The only difference in their treatment was the addition of defluorinated superphosphate to the drinking water of Group 4. The purpose of this was to ascertain whether the phosphate would counteract the effect of fluorine on the health of the animals. It appears, however, to have had exactly the opposite effect on reproductive ability. In this connection reference should be made to the statement by Mitchell & Edman (1952) that feeding of partially defluorinated phosphates constitutes one of the hazards of fluorine to livestock, and Truter & Louw (1952) admit that defluorination by the method they described was only partial. A logical conclusion, therefore, is that the fluorine intake of Group 4 might have been more than 8 ppm.



FIG. 1 .--- Calving rate of cows on three levels of fluorine intake

The calving rate of the animals on the three different levels of fluorine intake is illustrated in Fig. 1. In the first breeding season which started when they had already been receiving fluorine for nine months, the calving rate was completely normal. In the second season it increased from $84 \cdot 2$ to $89 \cdot 5$ per cent in the 5 ppm group, but decreased sharply from 85 to 60 per cent in the 8 ppm group, while the 12 ppm lot showed an even more pronounced decline from 90 to 40 per cent.

After the second calving reproductive efficiency dropped rapidly with an almost linear decline in all three lots and their calving rate in the fourth breeding season reached the abnormally low levels of $36 \cdot 8$, 30 and 10 per cent respectively.

The exceptional decline in fertility is in direct contrast with the findings of Van Rensburg & De Vos (1962) for normal animals. Working on the same breed and on the same farm they established that reproductive efficiency in the Afrikaner increases progressively from the third to the tenth year and over.

 TABLE 2.—Frequency of calving and total calf production in four breeding seasons by the individual groups on three levels of fluorine intake

Group	No. of ani- mals	Number of animals in each group that calved			Total	Calving	
Group		Four times	Three times	Twice	Once	produced	centage)
5 ppm fluorine 8 ppm fluorine 12 ppm fluorine	19 20 10	41	7 8 1	6 7 3	2 4 6	51 46 15	67·1 57·5 37·5

Table 2 gives an indication of the regularity, or rather irregularity, characterizing conception and calving by the cows on the three levels of fluorine intake. It shows for instance that only five of the 49 calved in every one of the four breeding seasons, and four of these belonged to the 5 ppm and one to the 8 ppm fluorine groups. In the other extreme six of the ten animals with a fluorine intake of 12 ppm produced only one calf each in the four years. Five of these calved as heifers in the first season and one in the second. Only one of the ten in this group produced more than two calves in the four years.

DISCUSSION

The data on the breeding history of the 49 animals in this experiment provide convincing evidence that the excessive intake of fluorine through the drinking water impairs breeding ability in cows. The results further agree with the finding of workers on other aspects of fluorosis, viz. that except when massive toxic doses are administered, the effects are cumulative and only manifested after continuous consumption for a year or longer. The intake of fluorine for nine months before the first breeding had no immediate adverse effect on the oestrous cycle, conception and pregnancy in heifers. Actually the group with the highest fluorine intake (12 ppm) put up the best breeding performance in the first season. Thereafter, however, the fertility of this group deteriorated rapidly.

The records of the group receiving 8 ppm also prove conclusively that the consumption of fluorine on this level in the drinking water will lead to serious interference with reproduction though the decline is not as rapid as in the higher intake lot.

Some caution must be exercised in evaluating the breeding records of the animals that received 5 ppm fluorine, since their conception rate was good throughout, not exceeding two services per conception in any season. The latter fact suggests that at no time was there any serious interference with fertilization in those animals that did show normal oestrus and were served. The marked drop in the calving rate of the 19 cows in this lot during the second half of the investigation, however, is ample evidence of undue inhibition of ovarian activity and of the onset of oestrus after calving. The evidence adduced justifies the conclusion that for normal reproduction the fluorine content of the drinking water should be under 5 ppm.

The sequence in which the physiological aberrations in the genital organs of the experimental animals appeared was as follows: firstly an unduly long suppression of ovarian activity after calving, the ovaries being in a static condition and feeling smooth and firm on rectal palpation. Thus 13 of the 30 heifers in the groups receiving 8 and 12 ppm, remained in this state so long after their first calving that they could not be served in the second season. Next to be observed during the succeeding breeding seasons was that in an appreciable proportion of those that did come into oestrus and were served, normal ovulation did not occur. In this connection the high incidence of anovulatory oestrus in Group 5 is significant. Finally it was evident that in some of those cows that did ovulate normally there was some or other impediment to fertilization at present unknown. This resulted in a very pronounced dropin the conception rate of the two higher fluorine intake groups during the third and fourth seasons.

It is significant that evidence of interference with reproduction was shown long before there were any symptoms of ill health, inappetence or mottling of the teeth.

Proof of impairment of breeding ability by the long continued intake of fluorine in amounts of 5 and more parts per million in the drinking water requires elucidation of the mechanism whereby the harmful effects are produced.

Those workers who did report adverse effects on reproduction, tend to the view that this is brought about indirectly through reduced feed intake, anorexia and the other adverse effects on the teeth and general health of the animals. It is understandable that ovarian activity and the oestrous cycle will be suppressed in animals suffering from chronic fluorosis, malnutrition and poor health. In this investigation, however, not one of the animals showed signs of poor health or loss of appetite up to the end of the fourth breeding season, though erosion, pitting and mottling of the teeth became increasingly evident. Furthermore the data on body weights furnished by Myburgh (1965), show that with one exception there was a steady increase in body weight till the conclusion of the experiment. The exception referred to is Group 3 which showed an average loss of weight of $5 \cdot 9$ Kg in the last year. Yet the fertility of this lot was not affected to any greater extent than that of any of the other

groups. Since, therefore, general health and bodily condition cannot be incriminated it can be postulated that the harmful effects on fertility result from direct adverse influence of excess fluorine on the endocrine organs that control reproduction, and that the thyroid is probably the gland primarily affected.

Steyn (1949) states that the incidence of goitre in human beings in endemic fluorosis areas in South Africa is above average and that there appears to be no doubt that the action of fluorine is antagonistic to that of iodine and thyroxine. For that reason he considers that the prevalence of goitre in the endemic goitre areas can be attributed, not to an exogenous iodine deficiency in the food and water, but to an endogenous deficiency of iodine caused by excessive intake of fluorine. Wilson (1941) found that the distribution of endemic goitre in the Punjab and in England is related to the geological distribution of fluorine and to the distribution of human dental fluorosis. Blood & Henderson (1963), too, point out that an excessive intake of fluorine has been suggested as a conditioning factor in the development of hyperplastic goitre, but that evidence on this point is conflicting.

That the thyroid is intimately connected with reproductive efficiency is well known. Hafez (1962) for instance states: "It is quite apparent that normal functioning of the thyroid gland and proper secretion of its hormones, thyroxine and triiodothyronine are a prerequisite for good reproduction". Pitt-Rivers & Trobler (1964) point out that in the spontaneously ovulating mammals hypothyroidism results in prolonged di-oestrus or even anoestrus, and there is a possibility that decreased gonadotrophin secretion from the hypothyroid animal's pituitary could lead to disturbances in the oestrous cycle. It follows accordingly that excessive intake of fluorine may spark off a chain reaction commencing with interference with uptake of iodine by the thyroid whereby thyroxine secretion is decreased, this in turn retarding or inhibiting the secretion of the gonadotrophins by the anterior pituitary. Ultimately static ovaries, anoestrus, anovulation and prevention of fertilization are produced, resulting in reduced fertility or even complete sterility.

Several workers have pointed out that the nutritional level of animals may have an influence on the severity of the symptoms produced in fluorosis. The animals in this experiment were maintained entirely on veld grazing and consequently the food intake was below borderline for six months of the year. This might have produced depression of fertility to an extent that may not occur in animals on a well balanced ration. Nevertheless, these conditions apply in practice to many of the ranching areas in South Africa and it is known that reproductive efficiency in those areas is poor, mainly on account of long periods of anoestrus after calving. It may be more than mere coincidence that the regions where the breeding performance is poor are those where the fluorine content of the soil and water is above average. The danger to stock in those areas is aggravated by the fact that on account of the low rainfall the drinking water is almost exclusively derived from underground sources, mainly from boreholes.

The indications are, therefore, that excess fluorine in the drinking water may play an important role in depressing fertility, especially in ranching stock in many parts of South Africa and it presents a problem which should receive high priority in any detailed investigation into herd infertility that may be planned for the future.

SUMMARY

Observations were made over four breeding seasons to determine the effect of excessive intake of fluorine in the drinking water on the breeding efficiency of cattle.

Fifty Afrikaner heifers, maintained under ordinary ranching conditions, were divided into five groups which received 5, 8 and 12 ppm fluorine respectively in the drinking water.

They were tested daily for oestrus during the breeding season (September to May) and those showing heat were served.

The two criteria for judging breeding performance were calving percentage and the number of services per conception.

In the first season reproduction was normal in every respect in all groups, but in the next season there was a noteworthy increase in post-calving anoestrus in the groups receiving 8 and 12 ppm fluorine. The third season revealed an appreciable decline in fertility, notably in the animals receiving over 5 ppm fluorine. The fourth season was characterized by a marked drop in breeding efficiency as judged by calving rate and services per conception in all groups. This was most pronounced in the groups receiving 8 and 12 ppm.

The addition of defluorinated superphosphate to the drinking water did not diminish the harmful effect of fluorine on reproduction, but, on the contrary, appeared to aggravate it.

The adverse influence of excessive fluorine on reproduction was manifested before the animals revealed any evidence of impairment of general health, such as loss of condition and inappetence.

It is concluded that for normal reproduction the fluorine content of the drinking water should be under 5 ppm.

The possibility that excessive intake of fluorine may inhibit thyroxine output, thus interfering with the normal secretion of gonadotrophins and giving rise to the functional disturbances observed in the ovaries, is discussed.

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