CHAPTER III

RESULTS AND DISCUSSION

This investigation concerns the effect of obesity on the performance of certain animals and the results to be reported on should exclusively pertain to the subject.

Incidental to the collection of relevant data however, and particularly during the earlier stages in the development of the heifers, certain observations were made e.g. in relation to the ovulatory performance, the relative roles of the right and left ovaries, the corpus luteum, cycle length, estrus, calving performance etc. which, although they have no direct bearing on the subject under discussion, afford valuable information on certain aspects of the functional development and reproduction of the young bovine.

It was decided not to omit such information, but to present it in entirety together with the direct subject matter of this study.

3.1 Ovarian Activity

3.1.1 Ovulation and ovulatory failure

The ovulatory performance of the heifers before they were inseminated and then after calving on the high and low feeding levels, are summarized in Tables 1(a), 1(b) and 1(c) respectively.

From Table 1(a) it can be seen that as heifers only two of them, numbers 2 and 30, exhibited normal ovulations with every cycle until they conceived, totals of six and seven cycles respectively.

Eighteen out of the 32 heifers, (56 per cent) exhibited normal ovulations in more than one half of their recorded estrus periods.

One heifer, number 23, never ovulated for 7 successive cycles before she went into anestrus for 261 days, during which period she developed follicular cysts in both ovaries. She started cycling again at irregular intervals but never conceived and developed heavy masculine features as a sterile animal.

TABLE 1(a) – Ovulation record of 32 Friesland heifers during all cycles from the onset of puberty until conception

Heifer Vers	Cycle No./Siklus Nr. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	0	0	0	θ	+	+	+	+				Normal ovulation (Ŧ)										
2	+	+	+	+	+	+	+	+				Delayed ovulation (θ)										
3	+	+	+	θ	–	+	+					Anovulation (0)										
4	θ	+	+	0	+	+	+	+				Abnormal cycle length (–)										
5	+	+	+	+	θ	+	θ															
6	+	–	θ	+	+	θ	θ															
7	+	+	θ	θ	+	+																
8	θ	+	+	+	θ	θ	+	+														
9	+	0	θ	0	+	–	+															
10	+	–	+	θ	+	+	–	–	θ													
11	0	0	0	0	θ	θ	θ	–	+	+	0	θ	+									
12	0	0	0	+	0	+	+	+	0	0	θ	+										
13	0	–	0	0	0	θ	+	0	+	0	+											
14	0	–	–	+	+	+	+															
15	0	0	+	+																		
16	+	0	0	+	+	+	θ	0	θ													
17	0	0	0	–	–	+	θ	θ	+	+	–	–	0	+								
18	+	+	–	+	–	+	+	θ	0	θ												
19	+	+	+	+	0	+	–	θ	–	–	–	–	–	0								
20	+	0	+	0	+	θ	θ	+														
21	0	+	–	+	0	θ	θ	+														
22	+	0	–	+	–	–	0	0	+	+	+	0	θ	+	0	θ	+					
23	0	0	0	0	0	0	0	–	–	–	–	–	–	–	–	0						
24	θ	θ	θ	0	θ	θ	+	+	+	θ												
25	0	0	–	+	+	0	+	+														
26	+	θ	+	+	+	+	+															
27	+	+	+	–	0	+	0	+	+													
28	0	+	+	+	+	+	0	+														
29	0	–	0	θ	+	+	+	+														
30	+	–	–	+	+	+	–	+	+													
31	+	–	0	0	+	+	+	+	+	0	+	+	+	+	+	–	0	0	–	–	+	+
32	+	+	–	θ	+	+																

TABLE 1(b) – Ovulation record of a group of Friesland cattle kept on a low feeding level (LOW group) after their first calving

LOW group																							
1	+	+	0	+	–	θ	0	+	+	+	+	+	+	θ	+	+	+						
3	–	+	+	+	+	–	+	+	+	+	+	+	θ	θ	+	+	+	+					
4	–	+	+	+	+	+	–	–	+	+	+	+	+										
6	+	+	–	0	–	+	+	+	+	+	+	+	+	+	θ	+	+	+	+	+			
8	–	θ	0	–	0	0	θ	+	0	θ	θ	+	0	0	+	+							
10	+	θ	θ	0	–	+	+	+	θ	+	θ	+	+	+	θ								
13	+	θ	+	θ	+	0	0	0	0	0	0	–	0	–	θ	θ	+	+	+				
16	θ	+	+	θ	+	–	θ	+	+	+	+	+	+	+									
20	–	+	+	+	+	0	θ	+	+	+	+	+	+	–	+	+	+	+					
21	+	+	+	+	+	+	+	+	+	+	+	+	θ	θ	+	+	+						
27	+	–	0	–	θ	+	+	–	+	+	+	θ	θ	θ	θ	+	+	+					
29	θ	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+						

TABLE 1(c) – Ovulation record of a group of Friesland cattle kept on a high feeding level (HIGH group) after their first calving

HIGH group																							
2	+	+	–	+	+	+	+	+	–	+	+	+	+	+	–	0	0	+	+				
5	+	0	+	+	+	+	+	+	+	+	0	θ	+	0	0	+	+						
7	+	+	+	θ	+	+	+	+	θ	θ	+	+	+	–	θ								
9	+	–	θ	θ	+	+	+	+	+	–	+	+	+	θ	+	+	–	+	+	+			
12	+	+	+	–	+	–	+	+	θ	–	0	+	+	–	0	0	–	0	–	θ	+	+	
14	θ	0	θ	+	+	+	+	+	+	+	0	θ	θ	0	+	+							
*	25	25		25		25		25		29		22		26		24		28		25		28	
17	+	+	+	+	+	θ	θ	+	+	+	θ	+	+	+	0	+							
18	+	θ	+	+	+	–	θ	+	+	+	+	+	+	+	+	+	+	+	+				
24	+	–	θ	θ	–	0	+	θ	θ	+	θ	θ	θ	–	0	θ	+						
25	+	+	+	+	+	+	0	–	θ	+	+	+	θ	+	+	+							
30	+	+	+	+	+	θ	+	+	+	+	+	0	–	θ	θ	+							
32	+	+	–	θ	0	+	+	+	0	–	0	+	θ	+	θ	0	+						

Normal ovulation (+) Anovulation (0)
Delayed ovulation (θ) Abnormal cycle length (–)

*13 out of 14 cycles prolonged

A further three heifers, numbers 19, 23 and 31, never conceived on repeated inseminations and maintained their relatively high rates of ovulatory failure until they were slaughtered as functionally sterile animals.

In heifers number 13 and 24 the ovulatory performance was well below the average for the group with seven ovulatory failures each in ten cycles. No apparent long term effects were, however, obvious in their fertility. Heifer 13, in particular, was recorded as highly fertile at the age of 5 years and at her third conception.

More unusual features were recorded in some of the heifers.

Number 4 suffered spontaneous haemorrhage on the right ovary at estrus. An adhesion slightly bigger than a hen's egg developed in which the ovary became embedded. Cyclic activity proceeded uneventfully with the left ovary being entirely responsible for all activity. In three out of 18 estrus periods, follicular development occurred in the right ovary in the adhesion. This heifer conceived with a single insemination each time on three different occasions.

Heifer 22 likewise suffered a haemorrhage in her right ovary at estrus and it became embedded in an adhesion larger than a mans fist. In this heifer however, cyclic and ovarian activity were impaired before the haemorrhage occurred and she remained sub-fertile afterwards.

Heifer 11 ovulated normally at only 3 estrus periods out of a total of 13 cycles, with delayed ovulation 5 times and anovulation 3 times. After the second cycle her right uterine horn increased in size until it was as big as a 3 months pregnancy but hard and solid on palpation. After 9 weeks during which she exhibited successive anovulatory estrus periods, the condition returned to normal spontaneously. She conceived only at the 5th insemination, failed to cycle for 80 days after calving, developed cystic ovaries and was recorded as sterile.

Heifer 14 had one anovulatory estrus period and then her right ovary became cystic and developed to the size of a golf ball for a period of 9 weeks, during which her uterus also enlarged to the size of a three month pregnancy like heifer 11. She recovered spontaneously and cycled again after 66 days, completed 5 normal cycles and conceived on a single insemination. She failed however to conceive after calving, and developed a cystic endometrium.

After calving, normal ovulations and the incidence of anovulation, delayed ovulation and abnormal cycle lengths were compared between the heifer stage and the combined group as adults and this is illustrated in Figure 1(a).

The ovulatory record of the HIGH and LOW feeding groups respectively, together with a comparison of their performance as heifers, are illustrated in Figure 1(b).

Heifers that were not allocated to the HIGH and LOW feeding groups, were also not taken into account in Figures 1(a) and 1(b).

If one argues that abnormal cycle length is the result of endocrine imbalance and further that anovulation is the result of a more severe imbalance than delayed ovulation, it appears from Figures 1(a) and 1(b) that adulthood after calving was characterized by a less precarious hormonal balance because of the considerable decrease in the percentage of cycles of abnormal length and because of the relatively decreased incidence of anovulatory estrus.

A comparison of the performance of individual heifers before and after calving confirms this as their ovulations in terms of the relative incidence of normal ovulations, anovulation, delayed ovulation and abnormal cycle length, improved considerably in 14 of the heifers. Slight deterioration was recorded in 9 heifers and one heifer remained exactly the same.

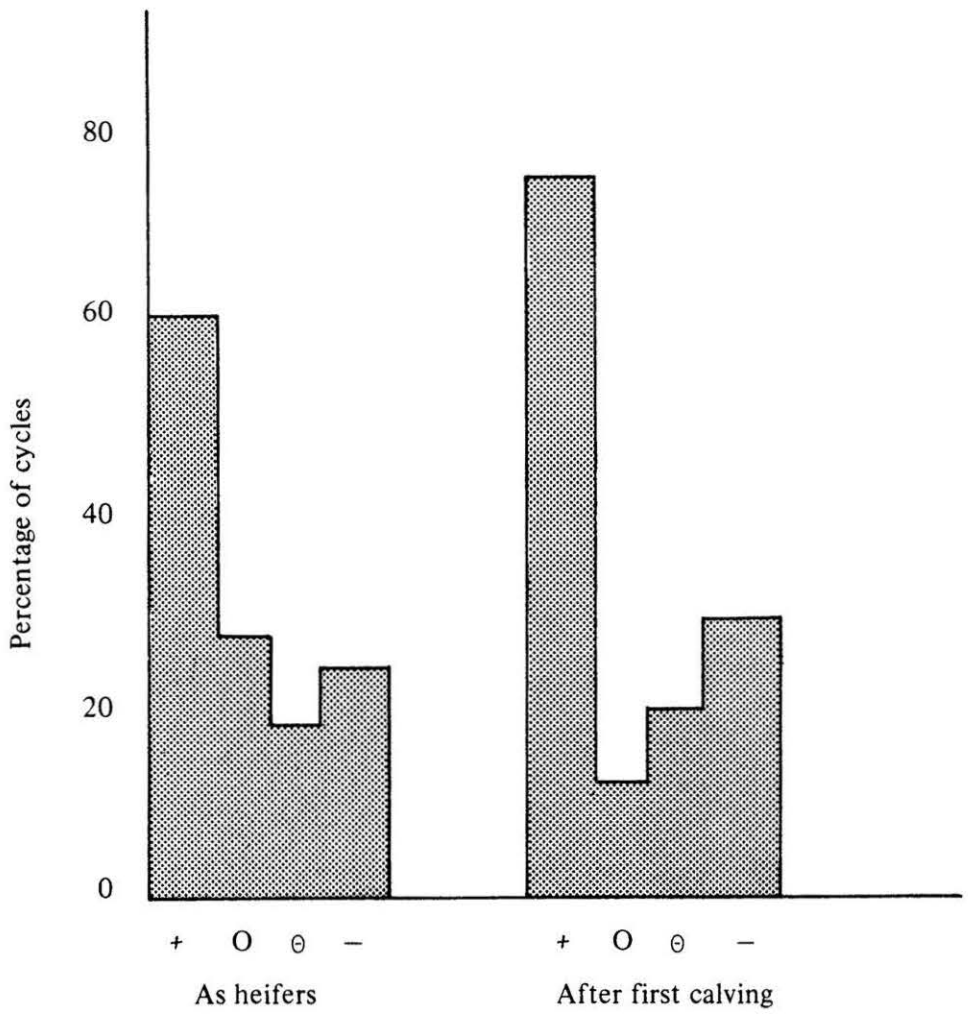
There were only minor differences if the performance of the heifers that were allocated to the HIGH group is compared to the heifers that were allocated to the LOW group (Figure 1(b).

After calving it is evident that the incidence of anovulation had decreased according to Figure 1(b). Delayed ovulation remained virtually unchanged and abnormal cycle length increased somewhat after calving.

A comparison between the LOW and HIGH groups in Figure 1(b) shows slight changes in favour of the LOW group where ovulatory failure and cycles of abnormal length were recorded less frequently. This will be illustrated in Figures 2(a) and 2(b).

It is evident that the delay of rebreeding after calving did not result in any obvious deterioration in the incidence of ovulatory failure in either the HIGH or

FIGURE 1(a) – Ovulatory performance of Frieslands dairy animals before and after calving



\+ Normal ovulations

O Anovulation

Θ Delayed ovulations

– Prolonged cycle length

FIGURE 1(b) – Ovulatory pattern before and after calving and on high and low feeding levels

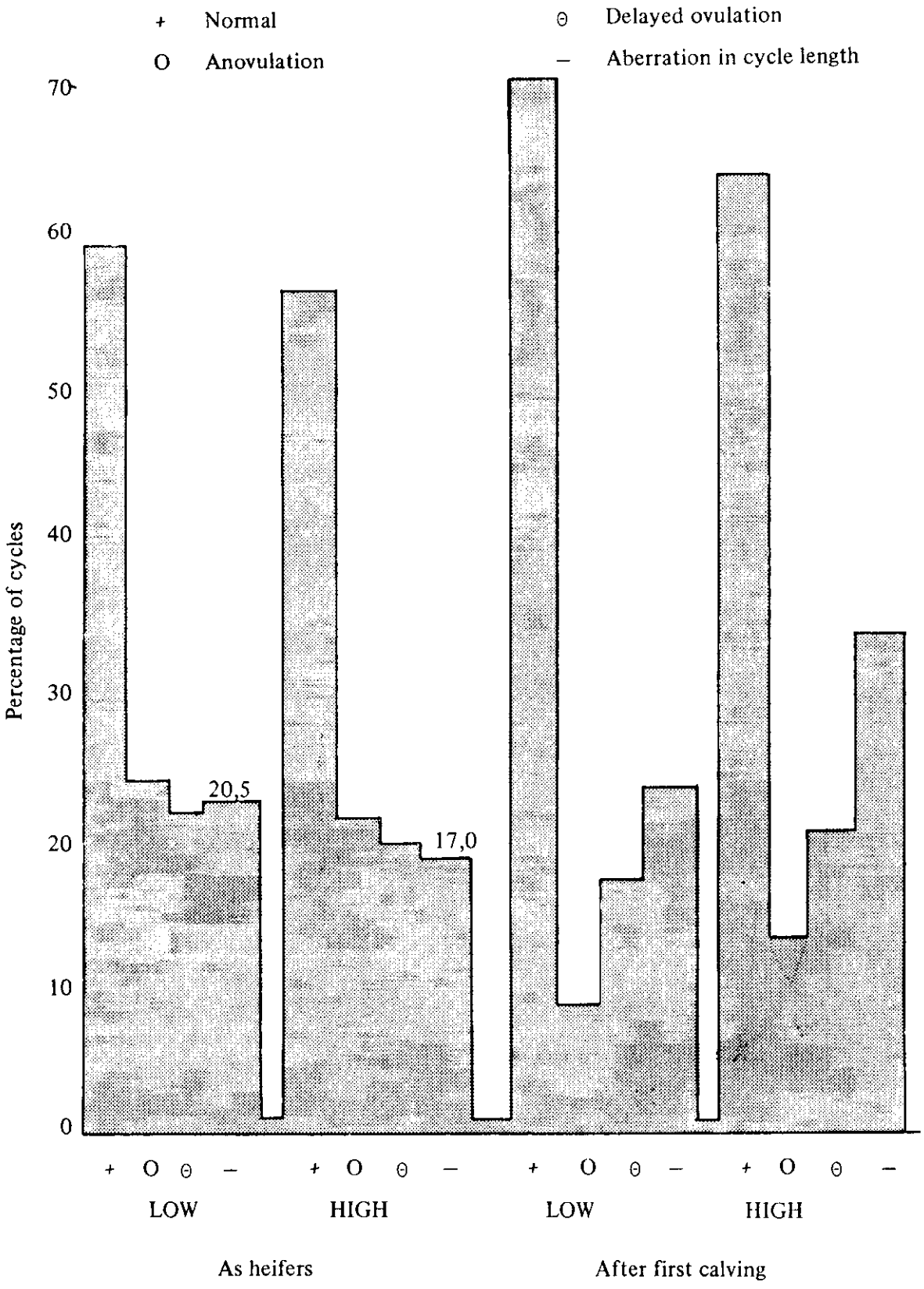


FIGURE 2(a) – Differences in cycle length before and after first calving and between HIGH and LOW groups after calving

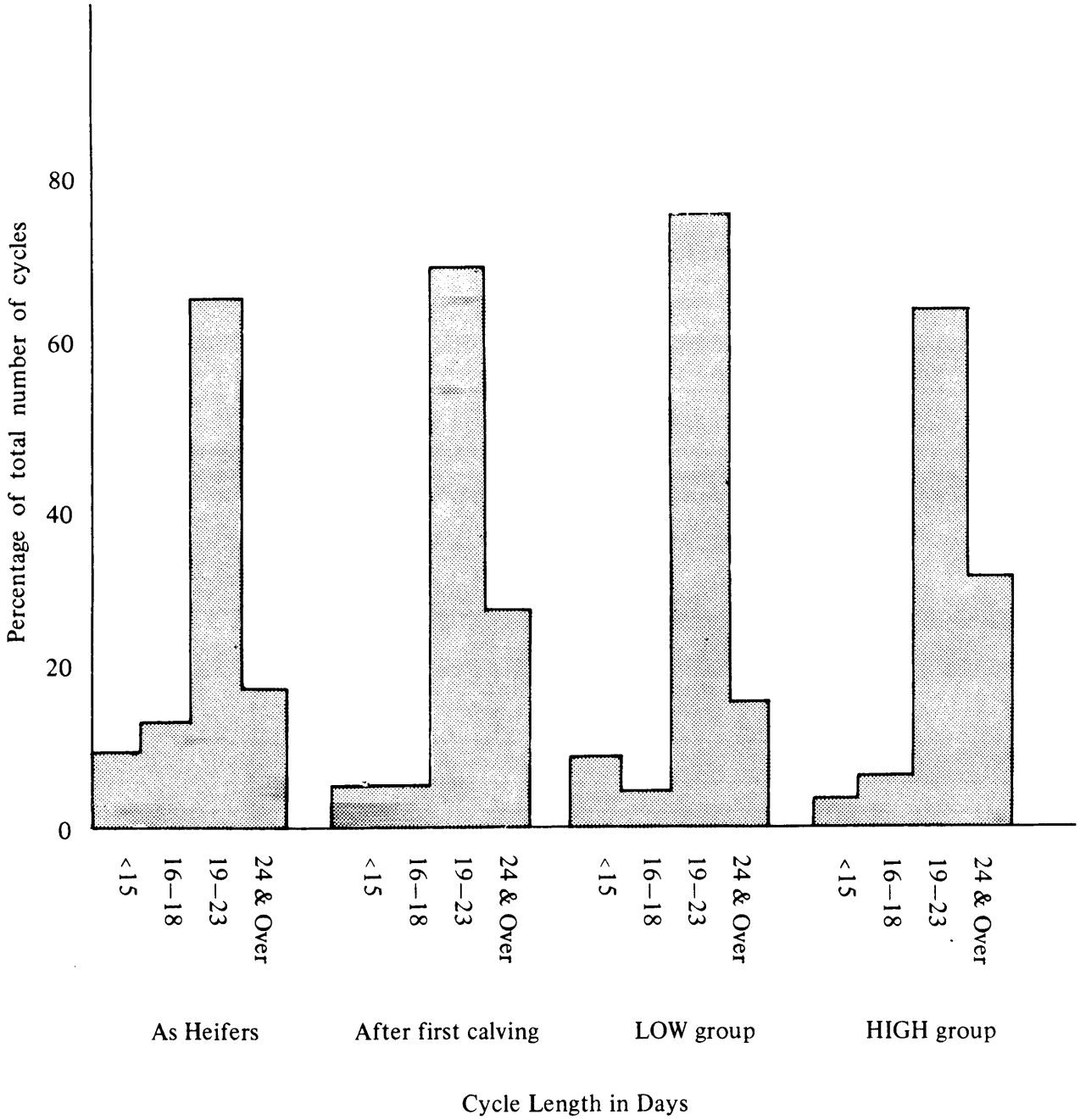
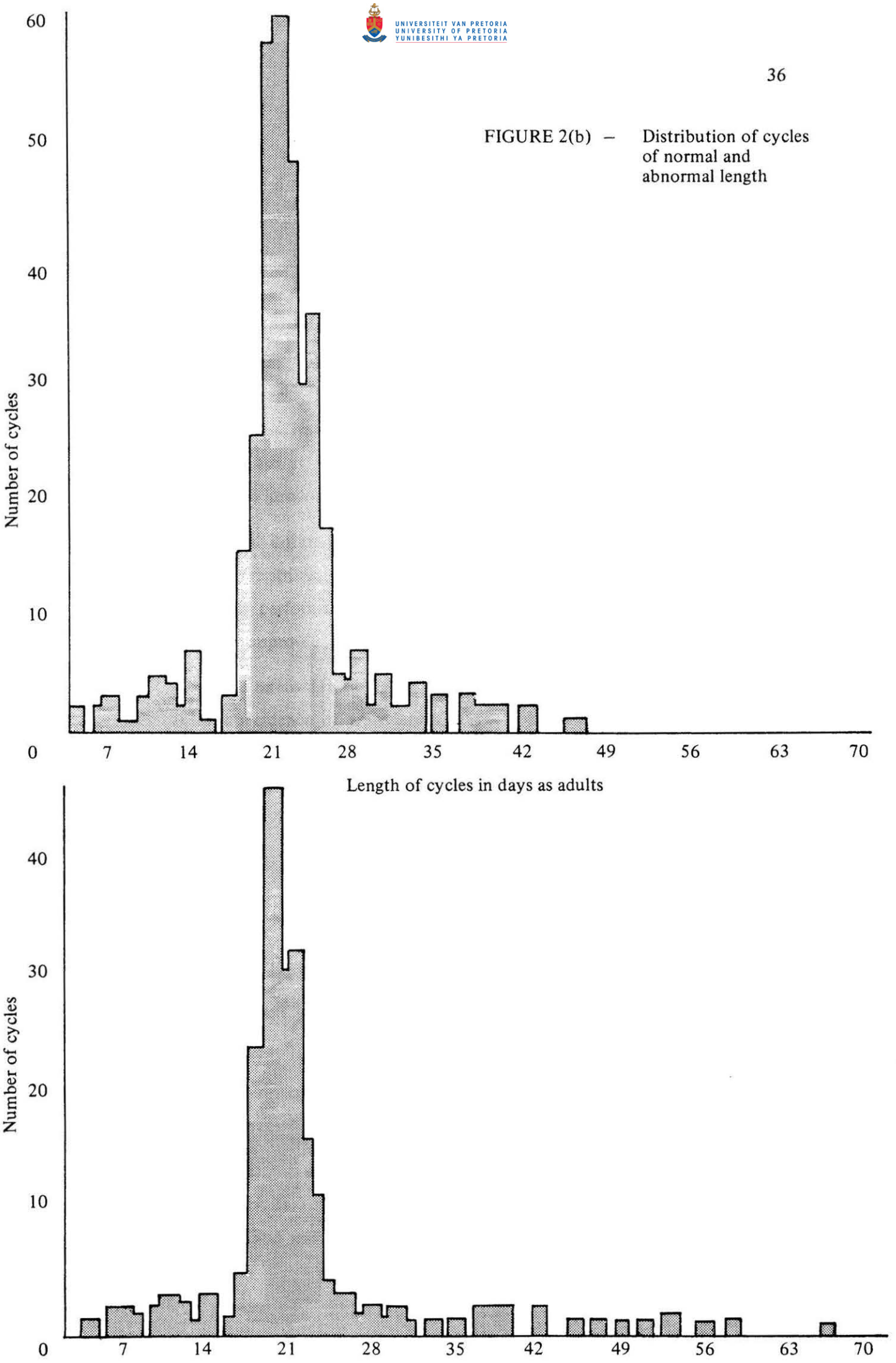


FIGURE 2(b) – Distribution of cycles of normal and abnormal length



the LOW groups. *Ad libitum* feeding however, was accompanied by a higher incidence of both ovulatory failure and cycles of abnormal length.

The overall picture showed clearly that functional disturbances in the majority of females improved with maturation in those individuals that appeared to be destined for a fertile life. In others again, maturation brought about severe deterioration in early aberrations so that the individuals concerned could readily be classified as sterile. *Ad libitum* feeding, operating through various pathways as will be shown, was responsible for a far more severe loss of animals from productivity at an age at which the dairy cow could be expected to attain a peak in her ability to produce and to reproduce.

Individual differences in ovarian activity during the heifer stage as well as after calving, were striking. Individual performances were reflected to a considerable extent by their performance as adults with the possible exception of 7 out of 24 animals which improved almost beyond recognition.

Reports on ovulatory failure in bovines are scant. Morrow (1969) recorded a 5 per cent incidence of anovulation in 53 Holstein heifers over their first three cycles with a significantly greater incidence at first estrus. He mentions a variable estrus behaviour at first ovulation. Standing estrus increased from 26,4 per cent at first ovulation to 79,3 per cent at the third ovulation.

Van Rensburg & De Vos (1960) examined the ovarian activity of Afrikaner cattle at estrus over 536 cycles and recorded ovulatory failure vis. delayed ovulation or anovulation in 140 cycles. They concluded that every cow shows ovulatory failure at some time during her breeding career.

3.1.2 Cycle length

The average length of all cycles, including cycles of abnormal length during the heifer stage was 21,8 days. If cycles of less than 15 days and over 25 days are disregarded, the average length of all normal cycles was 19,9 days.

As adults the overall average cycle length was 22,0 days. In the LOW group, cycle length was 21,0 days and in the HIGH group, 23,0 days. Disregarding cycles under 15 days and over 25 days, average cycle length was 21,0 days for each of the two groups.

The distribution of cycle length in the group as heifers compared to the total group after calving as well as in the HIGH and LOW groups respectively, are illustrated in Figures 2(a) and 2(b).

As heifers a larger number of short cycles were exhibited and as adults a larger number of longer cycles were exhibited according to Figures 2(a) and 2(b).

Out of a total of 268 cycles recorded, 137 cycles (24 per cent) were of abnormal length vis. shorter than 15 days or longer than 25 days.

A modal cycle length of 20 days was reported for dairy heifers and 21 days for dairy cows by Asdell (1946). Moeller & Vandemark (1951) reported that only 56 per cent of animals in their study of 4 885 cows had cycles of 18 to 25 days as determined by the interval between artificial inseminations. Asdell and his associates (1949) found that 16 per cent of cows showed cycles that were shorter or longer than 18 to 24 days, while Salisbury & Vandemark (1961) quoting Ellenberger & Lohmann (1946) report that 30 per cent of 274 cycles observed in cows occurred at intervals less than, or greater than, 17 to 25 days.

According to Cole & Cupps (1969) and Fallon (1958) cycles in cows shorter than average tend to be grouped between 8 and 10 days. In this investigation, they were grouped between 11 and 14 days (Figure 2(b)).

Moeller & Vandemark (1951) and Cole & Cupps (1969) presented further reports that cycles longer than 22 to 23 days tend to be grouped between 40 and 44 days which correspond to multiples of normal cycle lengths. This was not supported by data presented by Fallon (1958) who showed that prolonged cycles were rather evenly distributed between 28 and 42 days.

According to Figure 2(b) in this study Fallon's results were supported rather than the earlier results quoted. Prolonged cycles showed no tendency to correspond to multiples of normal cycles.

It further seems odd that cycles of 15 and 16 days were virtually not recorded in the heifers as well as in the adults.

During the heifer stage, cycle length was abnormally increased or decreased in 44 (14 per cent) of the 231 cycles recorded as against ovulatory failure which was recorded in 41 per cent of cycles.

As adults after the first calving, the position was reversed. In the LOW group 38 out of 168 cycles (23 per cent) were of abnormal length as against ovulatory failure in 14 per cent of cycles. In the HIGH group 55 out of 169 cycles (33 per cent) was of abnormal length with ovulatory failure in 20 per cent.

In 68 out of the 137 estrus cycles of abnormal length (50 per cent), cycle length was increased while a corpus luteum could be palpated on one of the ovaries. The other prolonged cycles all followed on anovulatory estrus periods and corpora lutea did not develop.

Follicular activity proceeded in one or both ovaries during long and short cycles.

The first two cycles after calving and the first three cycles after the onset of puberty, was characterized by a much higher incidence of aberrations in cycle length than the overall incidence.

The incidence of ovulatory failure at estrus following on cycles of abnormal length, is recorded in Table 2.

TABLE 2 – The incidence of ovulatory failure at estrus following on cycles of abnormal length

	Ovulatory failure in all cycles	Ovulatory failure in cycles of abnormal length
Heifer stage	41%	70%
Adult stage after calving		
HIGH group	20%	53%
LOW group	14%	42%

It is clear that the incidence of ovulatory failure at estrus following cycles of abnormal length, was higher than the overall incidence of ovulatory failure recorded. In the heifers, this was 70 per cent against 41 per cent, and in the LOW and HIGH groups, 42 against 14 per cent and 53 against 20 per cent respectively. The inference can be made that reduced fertility is associated with cycles of abnormal length.

At estrus periods following on cycles where a persistent corpus luteum could be palpated on the other hand, ovulatory failure was recorded in only 19 out of 86 cycles (22 per cent).

This is considerably lower than in estrus following the overall number of cycles of abnormal length and quite in proportion to the incidence of ovulatory failure recorded in cycles of normal length.

Evidence is available that the fertility of cows tend to be low after short di-estrus intervals (Moeller & Vandemark, 1951; Fallon, 1958.)

Salisbury & Vandemark (1961) quote Trimberger & Fincher who reported that in cows recovering from retained corpora lutea the conception rate was as high as that of normal cows, which substantiates the belief that no major disturbance other than delay of estrus is caused by persistent corpora lutea. They recorded 27,6 per cent of 500 cycles longer than 25 days and found that retained corpora lutea were responsible for over 40 per cent of these cycles or 12,5 per cent of the total number of cycles.

In the present study these findings were confirmed. Approximately 50 per cent of cycles of abnormal length were associated with a persistent corpus luteum and fertility appeared to have been unaltered. In the absence of a corpus luteum, subsequent estrus periods appeared to be characterized by a high incidence of ovulatory failure.

Abnormalities in LH and other endocrine levels are responsible for ovulatory failure but few factors have been recorded to influence the required levels of these hormones.

In a series of trials on the effect of luteinizing hormone on corpus luteum life-span, Wilks & Hansel (1971a), Swanson & Hafs (1971) and Wilks & Hansel (1971b) reported that elevated levels of LH were determined on days 3 and 4 of the cycle and that these levels represent a normal physiologic stimulus for maximal luteal development and increased corpus luteum function which occurs after day 4 of the cycle. The peak of LH secretion preceded standing heat by 3 hours, lasted for 6 to 8 hours and ovulation occurred 32 hours after the LH peak.

The incidence of cystic ovaries that will be reported on does not explain the much higher incidence of cycles shorter than 15 days. Neither were short cycles of less than 15 days or the presence of ovarian cysts on palpation, symptoms that were recorded in three cases where a cystic endometrium was found on histological examination.

The persistant and regular follicular activity apparent on palpation made it difficult to classify prolonged estrus cycles where no persistent corpus luteum was present, as non functional ovaries.

The incidence of ovulatory failure as well as the disturbances in cycle length demonstrated so far in this group of heifers, should be regarded as manifestations of the delicateness and precariousness of the overall hormonal balance responsible for bovine reproduction. In the abscence of any other known factor like environmental, nutritional or pathological conditions that might be responsible for the considerable individual differences between heifers, these aberrations should be seen as weaknesses in the inherent endocrinological faculties in some individual animals, a subject which will receive further thought at a later stage.

3.1.3 The time of ovulation from the onset of estrus

The time of ovulation from the onset of estrus is summarized in Table 3 and graphically illustrated in Figures 3(a) and 3(b).

Although the incidence of delayed ovulation appeared to remain unaffected by maturation from the heifer stage to the adult stage according to Figure 1(a) and 1(b) some improvement is evident from Figure 3(a) in the extent to which ovulation was delayed.

According to Figure 3(b), no difference is apparent between the HIGH and LOW groups.

The relation between the time of ovulation from the onset of estrus and fertility will be discussed further under conception in relation to ovulatory performance.

TABLE 3 — Time of ovulation from the onset of estrus

	Time (hours)	12–36	36–60	60–84	84–108	108–132	132–156	Anovulations
As heifers 259 Cycles	Number of cycles ovulated	147	20	10	7	3	3	69
	Percentage	59	8	4	3,1	1	1	24
As adults 374 cycles	Number of cycles ovulated	272	40	15	4	4	2	37
	Percentage	73	11	4	1	1	0,5	9,5
HIGH group 192 cycles	Number of cycles ovulated	133	21	9	3	2	1	23
	Percentage	69	11	4,9	1,7	1,0	0,4	12
LOW group 183 cycles	Number of cycles ovulated	139	19	6	1	2	1	14
	Percentage	76	10	3,3	0,7	1,5	0,7	7,7

FIGURE 3(a) – The time of ovulation from the onset of first estrus as heifers and after first calving

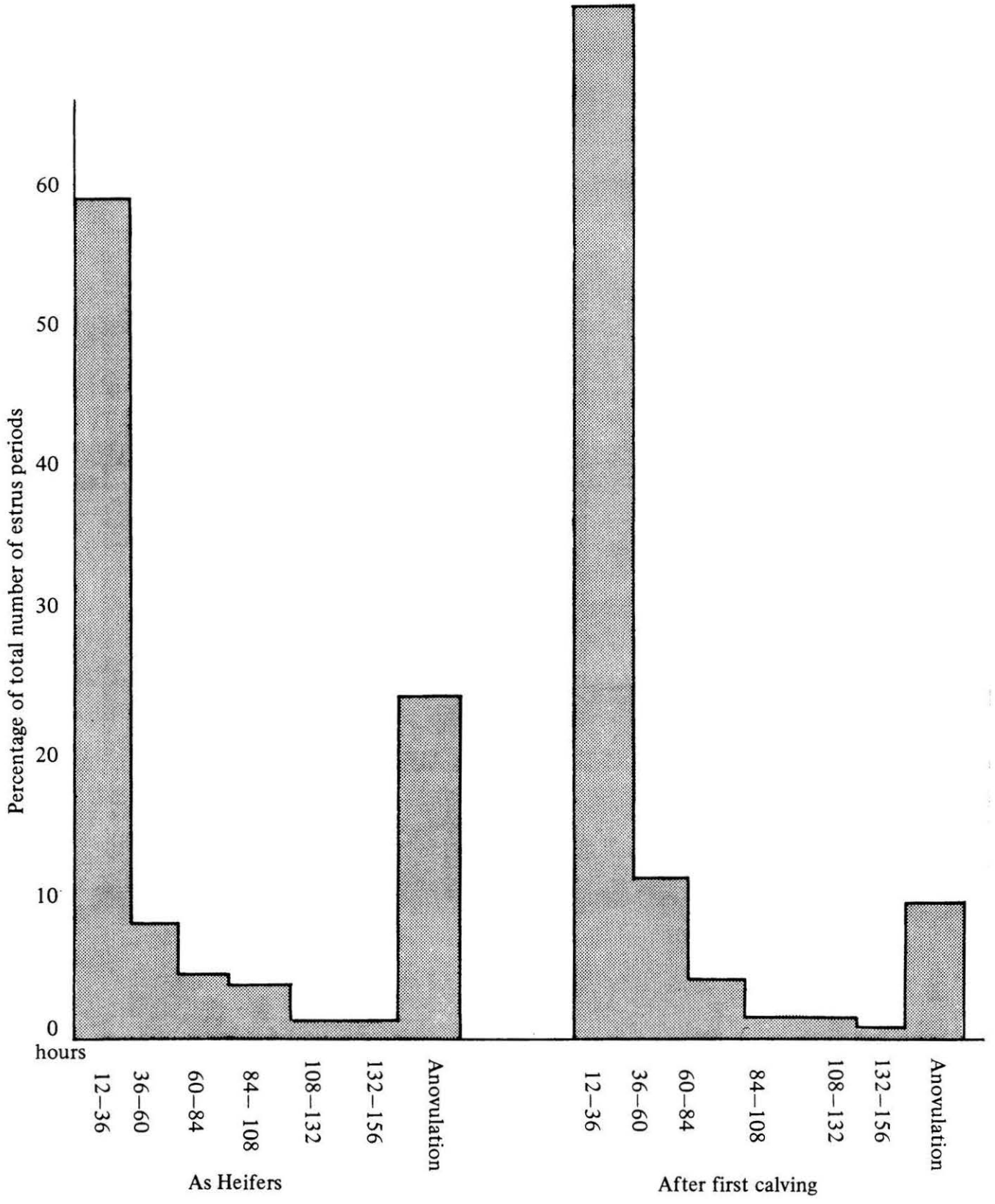
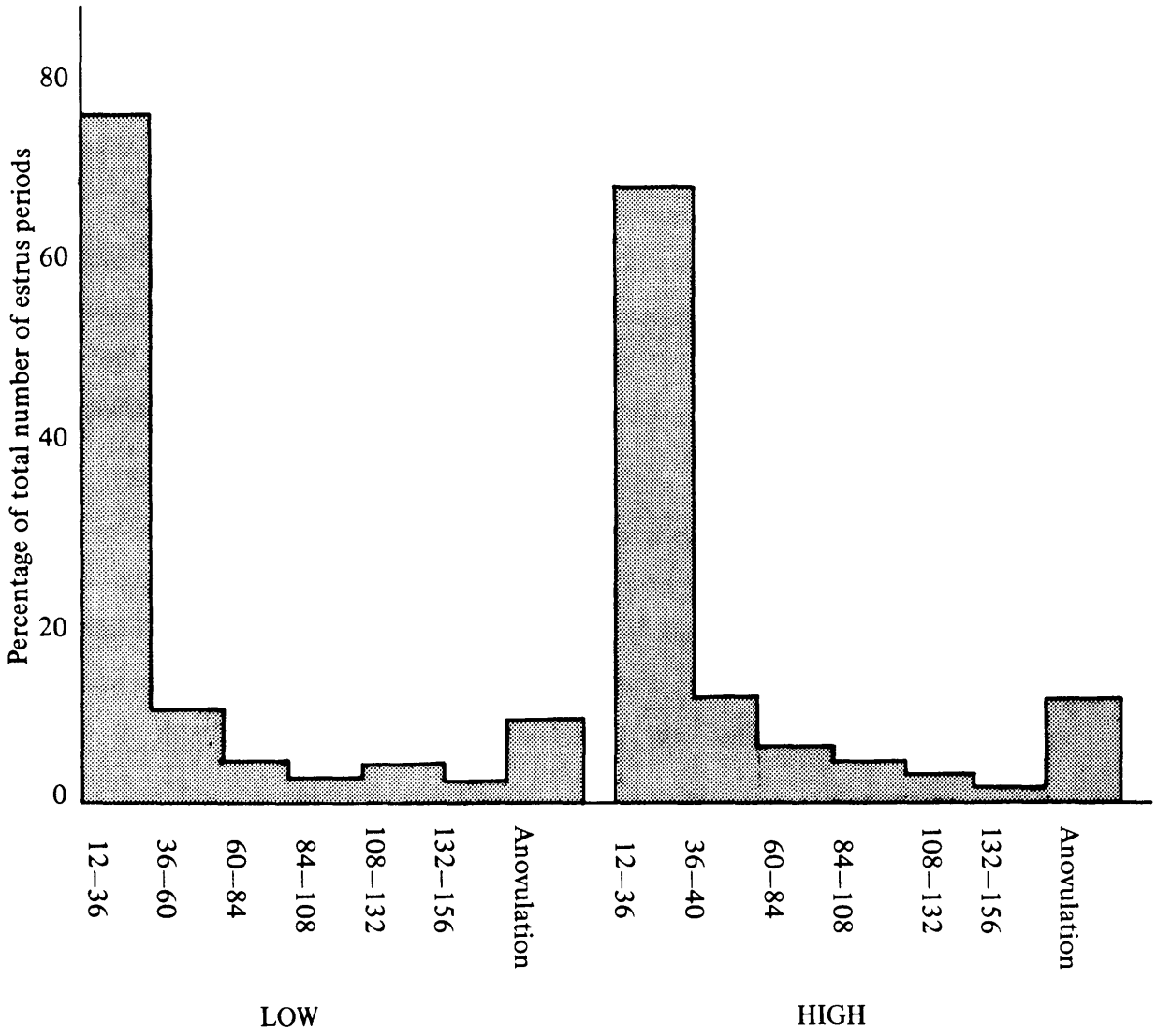


FIGURE 3(b) – The time of ovulation from the onset of estrus in cattle on a LOW and a HIGH level of feeding after first calving



3.1.4 Ovulation and estrus performance

It was possible on palpation to establish whether a follicle was ready to ovulate on the day of estrus. The ripe follicle was well defined but soft and thin walled and distinctly not fully distended with fluid.

On a number of occasions ovulation occurred at the time of examination while the ovary was being handled. A collapse of the follicle could be felt between the fingers as fluid oozed out rather slowly. An impression of follicular rupture with a rapid release of fluid was never gained such as when a follicle or cyst was purposely ruptured by hand.

In cases of anovulation or delayed ovulation, the Graaffian follicle was definitely less fragile. It was firm and more thick walled on palpation and never ovulated on handling. Ripening causes an increase in size as well as softening of the follicle as described by van Rensburg & de Vos (1960) and this was readily evident on palpation.

Edwards (1970) quoting Rondell reported a fall of intra-follicular pressure and he suggests enzymically induced changes in the follicular wall with alterations in its mechanical properties and a decrease in wall thickness. Cole & Cupps (1969) suggested that rupture under pressure is not the essential mechanism in follicular opening and that in most instances ovulation is a gradual process depending as much on progressive changes in the structure of the follicle wall as upon internal pressure.

Hafez (1962) and Cole & Cupps (1969) record normal ovulation time as from 2 to 26 hours after the end of estrus or 12 to 15 hours after the end of an 18 hours estrus respectively.

Salisbury & Vandemark (1961) summarized data presented by various workers over many years. With ovulation timed from the beginning of estrus, time of ovulation varied from 16 to 65 hours with an average of 25 to 30 hours after the onset of estrus. Other investigators again determined ovulation by using the end of estrus as a starting point and recorded ovulations to occur from 2 hours before the end of estrus to 26 hours after the end of estrus, the average time being 12,5 hours after.

The deviations recorded in this study in an average group of clinically normal heifers and again as adults should apparently be considered as the regular variation for normal cattle.

The starting point of estrus however was gradual and vague. Different manifestations of overt estrus were observed during the initial stages and they developed during a period which varied from one to several hours between heifers viz. the appearance of vaginal discharge, the mounting of other heifers and acceptability to other heifers and particularly the intensity of these manifestations as well as the intervals at which these manifestations were observed.

For the purpose of this study an observer spent his time very close to the animals from light until dark every day. He regularly reported excessive playfulness and advances of certain individual animals two days before they came into estrus. Some animals would frequently attempt to mount him on the day before any interest in or by other animals was shown.

Observation for the end of estrus was likewise hampered by the gradual and odd abatement of signs so that the exact end point was determined somewhat subjectively.

3.1.5 Follicular and corpus luteum development

Considerable variation was encountered in the size of the Graaffian follicles at estrus. It was a distinct feature for some animals to develop relatively small Graaffian follicles at estrus and for a few cycles in succession to be followed suddenly by estrus periods with much larger follicles.

Estrus behaviour, ovulation or ovulatory failure and fertility could not in any way be associated with follicular size at estrus. Relatively small follicles on palpation were found to ovulate readily at estrus, often rather against expectations, as against large well developed follicles which sometimes failed to ovulate.

Corpus luteum size as far as could be determined by palpation did not in any way seem to be determined by the size of the follicle from which it developed. Small follicles were often recorded and large corpora lutea developed from them and vice versa.

Considerable variation in corpus luteum size as well as shape and consistency was encountered on palpation and to quite a surprising extent with the same corpus luteum on successive examinations. A corpus luteum that felt firm and solid on one examination was often recorded as soft and flabby on the next examination, and could easily have been confused with a follicle or cyst had a series of examinations not taken place.

It was always possible and particularly easy in newly developed corpora lutea, to distinguish them from follicles or cysts by the typical crepitation of the corpus luteum on pressure. Neither did this practice ever lead to the accidental expression of a corpus luteum as long as pressure was applied to the tip and not the base of the corpus luteum that was embedded in the ovary.

On several occasions the tip of the corpus luteum was split on pressure but this never affected the life span of the corpus luteum.

Development of the corpus luteum was palpable from the second to the third day after ovulation and frequently crepitation could be felt at 24 hours after ovulation.

Mares, Zimbelman & Casida (1967) recorded that functional activity in the bovine corpus luteum increased to day 15 followed by a decline in progestogen concentration and content, RNA/DNA and per cent type I and II luteal cells. The abrupt drop in progestogen concentration from days 15 to 17, was followed by a lesser drop in corpus luteum mass. Corpus luteum mass increased sharply from day 5 to day 7 at which maximal mass was attained. At day three corpus luteum mass was of the order of only 20 per cent of maximal mass but progestogen concentration and RNA/DNA was fully established.

Cystic corpora lutea were a common feature particularly in the newly developed corpus luteum a few days after ovulation. Fluid escaped readily on mild pressure and there was a tendency for re-accumulation of fluid. In one heifer for example fluid escaped from the corpus luteum on three successive examinations.

The corpus luteum of pregnancy was cystic on several occasions during the early stages but without any apparent effect on the course of pregnancy. In one heifer however, a large cyst of $\pm$ 3 cm in diameter persisted on the corpus luteum

for 52 days after insemination when the heifer aborted. The cystic corpus luteum as a possible cause of the abortion could naturally not be confirmed.

The overall impression was, however, that fluid in a corpus luteum was incidental to its development and that it had no effect on the life span or function of the corpus luteum as far as could be discerned from this investigation.

There appears to be uncertainty on the functional efficiency of cystic corpora lutea from the results of various investigators. Roberts (1957) summarised various reports on cystic corpora lutea and concluded that they are uncommon in cattle and that they develop subsequent to ovulatory failure. The opinion is expressed that cystic corpora lutea may often be associated with prolonged periods of anestrus in cattle. The difficulty to differentiate between cystic corpora lutea and cystic follicles is mentioned.

Staples and his associates (1961) reported that cystic corpora lutea appear to produce little progesterone and that only an occasional cystic corpus luteum can maintain an embryo until the 15th day. Braden & Moule (1964) on the other hand stated that the presence of a cavity in the corpus luteum of sheep, seemed to have been induced by stress and that the amount of luteal tissue and its microscopic appearance did not seem to differ from solid corpora lutea.

Casida & Chapman (1951) reported that the incidence of cystic corpora lutea was correlated with the amount of handling that cows were subjected to.

A fairly constant progesterone content in cystic corpora lutea was reported by Donaldson & Hansen (1968) however, and they concluded that corpus luteum cysts can not be regarded as abnormal in cows. According to Marion & Gier (1968) too, cystic corpora lutea do not affect cycle length.

Morrow, Roberts & McEntee (1969) found no influence of the frequency of examination, level of production, parity and herd environment on the frequency of cystic corpora lutea in cattle, which in their studies did not have an adverse effect on pregnancy and disappeared during the first 2 to 4 months after conception. Cycle length was also found not to be influenced by cystic corpora lutea but season was, however, found to have a significant influence on the frequency of cystic corpora lutea, the mechanism of which was not explained by them.

Cystic corpora lutea in this study developed incidental to corpus luteum formation as mentioned and were not found to be associated with periods of anestrus as recorded by Roberts (1957), neither did anovulation induce anestrus as mentioned by him.

Prolonged cycle length or anestrus in this study followed on normal ovulations as readily as on ovulatory failure. A persistent corpus luteum was recorded, and was therefore a potential cause, only in approximately one half of the total number of cases of prolonged cycles that were recorded.

The impression is thus clearly gained, albeit speculatively, that two mechanisms were involved in cycles of abnormal length, one in relation to luteal life-span or luteolysis and the other in relation to the timing or the initiation of cyclic activity per se.

Luteinization of unruptured Graaffian follicles would naturally have escaped detection by rectal palpation, a problem also encountered by Morrow and his co-workers (1969).

The functional effect of luteinized follicular walls was neither obvious in any way. On palpation too, all unovulated follicles appeared to have regressed well within one cycle length and a smooth ovary of reduced size or new follicular development was palpated.

Follicular cysts were encountered in four of the heifers before they reached the age of 12 months. In each case the development of cysts was preceded by cycles of abnormal length and a high incidence of delayed ovulation or anovulation, while enlargement of the uterus followed two cases of follicular cysts in the heifers.

Nymphomania was never recorded at any stage.

After calving a cystic endometrium was recorded on histological examination in three cows, only one of which had developed ovarian cysts during the 5 years that they were under observation.

Callaghen and his co-workers (1971) emphasized the seriousness of ovulatory failure and abnormal cycle length in their report that early enlargement of ovaries and early growth of follicles with delayed rupture and decreased corpus luteum life-span characterized cows subsequently developing persistent large cystic follicles on the ovaries.

Jubb & Kennedy (1970) report that cystic endometrial hyperplasia in cows was invariably associated with ovarian follicular cysts which are causes of hyperestrogenism.

Donaldson (1969) again, produced cystic follicles in heifers with oxytocin and this was accompanied by hyperplasia of the endometrium. Polycystic ovaries also, may be associated with hyperfunction of the ardrenal gland (Najib Abu-Haydar, Laidlaw, Nusimovich & Sturgis, 1954).

Ovarian cysts are well recorded in bovines although there appears to be insufficient knowledge on the hormonal mechanisms involved. Evidence is available that cystic follicles occur more frequently in older and in high producing cows after the 2nd to 5th lactation than in heifers (Casida & Chapman, 1951; Salisbury & Vandemark 1961, quoting Garin; Wiltbank, Tyler & Casida, 1953).

Casida & Chapman (1951) reported an 18,8 per cent incidence of cystic ovaries in 341 cows at some time or other. Wiltbank, Tyler & Casida (1953) recorded a 20 per cent incidence and Trimberger & Fincher (1956) a 10 per cent incidence.

In many instances, cows with large unruptured follicles recovered spontaneously (Wiltbank, Tyler & Casida, 1953; Trimberger & Fincher, 1956), but 117 breeding days were lost in 341 cows through cystic follicles without signs of nymphomania, and 125 days in cows showing nymphomania.

These reports provide valuable information on the complexities and interrelationships of the various endocrines and their target organs, as well as on the incidence of some disturbances produced by these imbalances.

Of importance in this study however, is the undeniable fact that right from the onset of cyclic activity, some heifers distinguished themselves by obvious and persistent functional aberrations. Furthermore, it can safely be stated that, on any single examination of the internal organs or on inspection of these organs at slaughtering, these aberrations would have escaped detection.

The gap in the present state of our knowledge on the valuation of animals, particularly heifers, for optimal reproductive or functional efficiency, is clearly emphasized.

3.1.6 The respective roles of right and left ovaries

At estrus it was not unusual to encounter a well developed follicle in each ovary and without re-examination it would sometimes have been impossible to establish which one of the follicles was destined to ovulate.

After ovulation rapid or slow regression of the opposite follicle occurred. Ovulation of a follicle on each ovary was never encountered.

The distribution of Graaffian follicles between the right and left ovaries was 59,4 and 40,6 per cent, respectively, which corresponds with results recorded by other workers. (Clark 1936, Reece & Turner 1938, Kidder, Barrett & Casida 1952, Saidudden, Riesen & Tyler 1963, Wagner & Hansel 1969).

No feasible reason was ever suggested for the difference between the two ovaries.

The distribution of function between the two ovaries was odd and without any consecutive order. Ovulatory activity would occur in one ovary for one or more consecutive cycles and then suddenly and without any fixed order be changed over to the opposite ovary for one or more cycles. Ovulation has been found for example to occur consecutively for 8 and 7 cycles, respectively, in the same ovary and a maximum of 5 cycles were recorded where ovulations occurred consecutively in each ovary.

Kidder, Barrett & Casida (1952) tested the hypothesis that the function of each ovary was completely at random and concluded from their data that there is no apparent tendency for cows to have a systematic sequence of ovulations from one ovary to another. Neither did they in their investigation from 138 ovulations in the right ovary and 114 from the left ovary at which breeding occurred, established any differential fertility between the two sides of ovulation.

Apart from the development of Graaffian follicles at estrus, follicular development and atresia were recorded at all stages of the cycle in one or both ovaries.

A numerical index value of 1, 2 or 3 was given to small, medium or large follicles as recorded on palpation and an average value was calculated for each day of the cycle. Activity was recorded over a total of 374 cycles and a minimum of

5 examinations were carried out per cycle at intervals that varied between 1 and 7 days, but never exceeded 7 days.

In Figure 4(a) the follicular activity in both ovaries together for the HIGH and LOW groups respectively, is illustrated.

Figure 4(b) illustrates the activity in the ovulating and non-ovulating ovaries respectively for the two groups.

It is clear that the high level of nutrition stimulated follicular development on every day of the cycle in the ovulating as well as the non-ovulating ovary.

On day 1 of the cycle, that is the day after ovulation, follicular activity was at its lowest in the ovulating ovary in both groups while follicular activity proceeded at a low level in the non-ovulating follicles that were present next to the developing corpus luteum.

A higher level of follicular activity was recorded in the non-ovulating ovary than in the ovulating ovary on the day following ovulation.

In the LOW group follicular development appeared to have been inactive for the first few days after estrus but activity was apparent in the HIGH group during this stage.

The ovary to ovulate exhibited a gradual rise in follicular activity in both groups with pre-estrus acceleration in follicular development in the HIGH group taking place at a much earlier stage in the cycle than in the LOW group, the follicle often being well developed to medium or large size 6 to 8 days before estrus in the HIGH group.

In the LOW group follicular activity was only apparent in the ovulating ovary 2 or 3 days before estrus, and often only for 1 day.

The question arises whether the early development of the follicles plays a role in the fertility of the ova to be released.

On this matter Fugo & Butcher (1971) provide an answer after a series of trials with rats. They postulated that pre-ovulatory ripeness of the ovum plays a role in fetal loss. Delay in ovulation was shown to have a significant effect on fertilization rate and failure of implantation. The incidence of anomalies at the one

FIGURE 4(a) Numerical index value of follicular activity in both ovaries on each day of the cycle in cows on a high (H) and a low (L) level of feeding

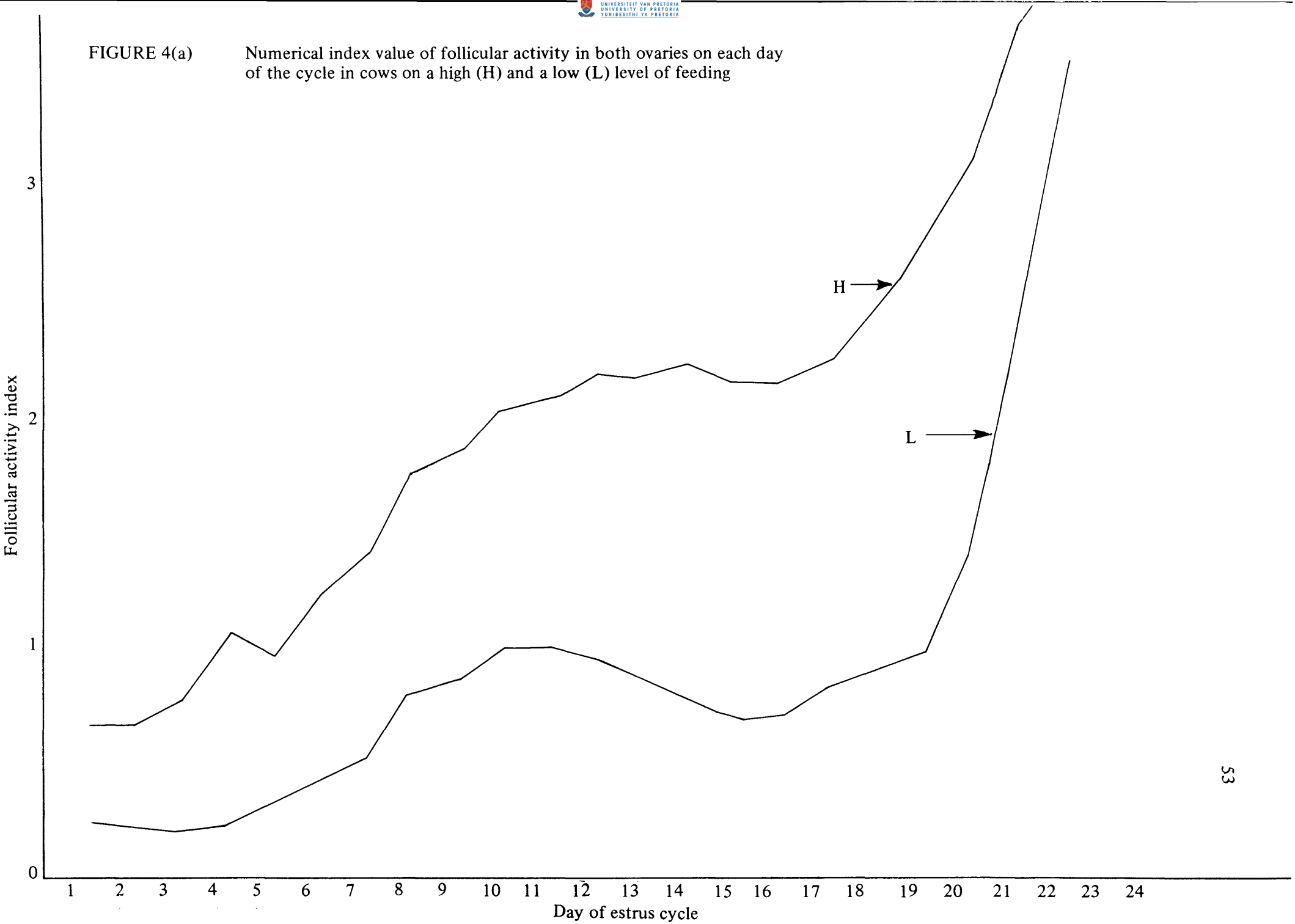
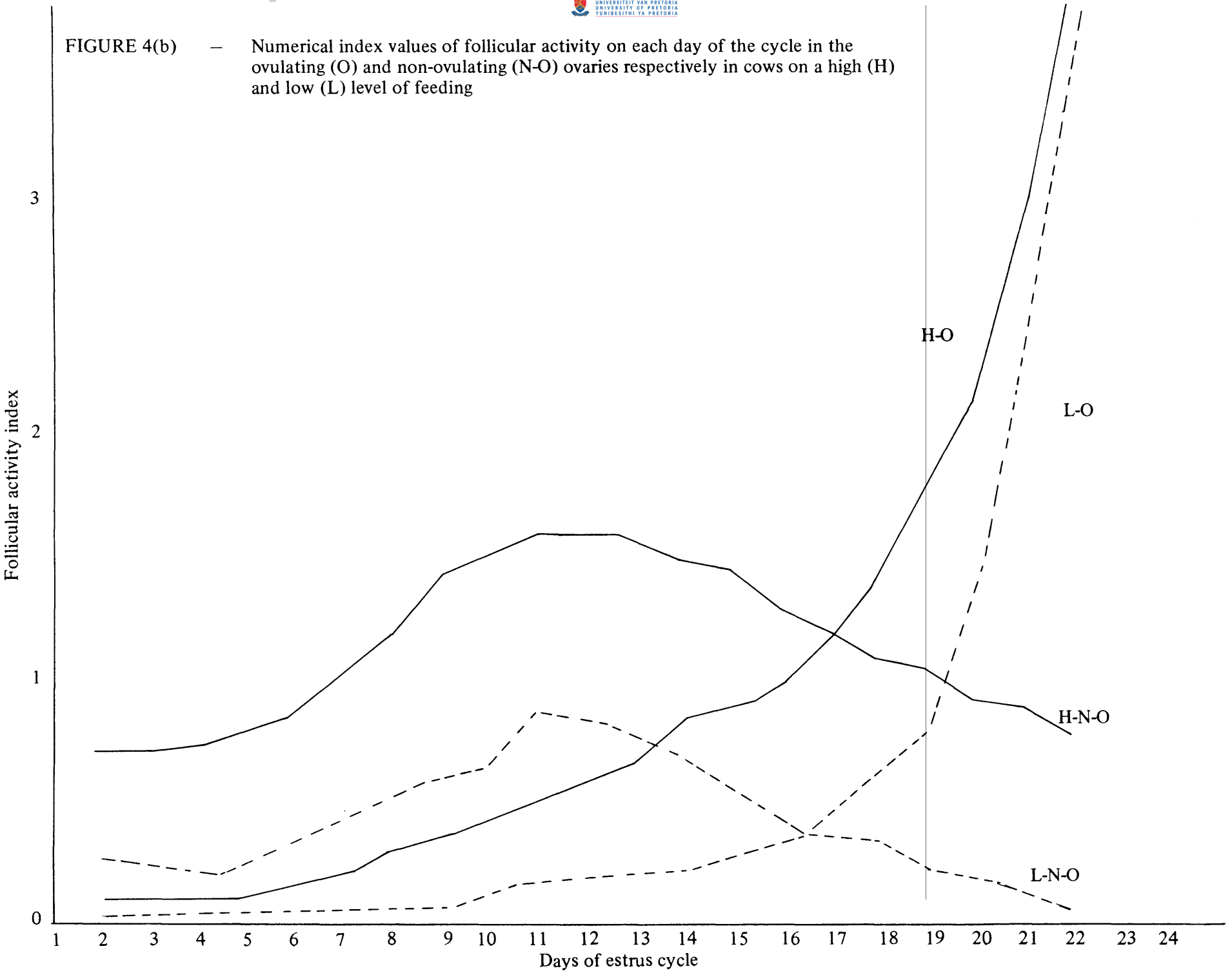


FIGURE 4(b) — Numerical index values of follicular activity on each day of the cycle in the ovulating (O) and non-ovulating (N-O) ovaries respectively in cows on a high (H) and low (L) level of feeding



cell stage of the ovum was shown to be greater from conceptions during a 6-day cycle than during a 4- or a 5-day cycle. Van Rensburg & De Vos (1960) again recorded normal fertility with delayed ovulations in cows where inseminations were carried out daily until ovulation. Ovum fertility in the bovine in relation to pre-ovulatory follicular development requires further investigation particularly since it has been shown so clearly in this study to be influenced by the level of nutrition.

Choudary, Gier & Marion (1968) showed that only a few hours are required to bring a follicle under 10 mm to ovulation size in the bovine. Their work also did not substantiate the view that the growth of follicles destined to ovulate occur in specific stages.

Two waves of ovarian follicles were reported by Rajakoski (1960). The first occurred between the third and the fourth day and the second between the 12th and the 14th day. The largest follicle of the first wave underwent atresia after the 12th day. The largest follicle of the second wave was ovulated.

On estrogenic function of these follicles, Rajakoski states that the period of 9 to 12 days is characterized by the secretion of large amounts of estrogen and progesterone, the impact of both of these hormones being essential in the preparation of the uterus for implantation.

According to Cole & Cupps (1969) cows showing strong response to weak doses of exogenous gonadotropic hormone, always have a large number of primordial follicles in reserve. Those cows that do not react to strong doses, have only small follicular reserves.

Rajakoski (1960) showed that heifers killed on the same day of the cycle differed greatly in total number of follicles >1 mm. On the second day for example, the numbers of normal and atretic follicles ranged from 26 to 127 and 35 to 139 respectively, and on the 20th day from 28 to 96 and 41 to 107.

He describes the effect of season while other workers (Riesen, Saidudden, Tyler & Casida, 1968) reported the effect of suckling but these reports fail to mention the importance of nutritional level indicated in this study.

The increase in follicular growth throughout the cycle in both ovaries in the HIGH group over the LOW group is most evident from Figures 4(a) and 4(b), while at estrus not much difference could be shown.

The wave of follicular activity reported by Rajakoski (1960) at mid-cycle is confirmed by Figure 4(a). Figure 4(b) shows, however, that this is brought about essentially through the activity of the non-ovulating ovary.

Cole & Cupps (1969) report a wave of follicular activity during the 3rd and 4th days of the cycle. This was confirmed in the present study, but only in relation to the HIGH group. In the LOW group, this wave of activity could not be recorded.

The wave of follicular growth reported by these workers between the 12th and the 14th day of the cycle and out of which a single follicle then remains to be ovulated, could also not be confirmed as the follicle to ovulate was shown to develop from the opposite ovary than the ovary responsible for this wave of follicles.

After fertilization, follicular development proceeded at a higher level in the HIGH group than in the LOW group. At the end of each cycle length, a wave of follicular growth was recorded in the HIGH group for the first two or three cycle lengths, after which follicular development ceased. This wave of follicular growth was suggestive of a "timing" mechanism for cycle length.

Summarily, it is suggested that the live animal forms a better subject for the investigation of follicular growth than the examination of organs at slaughtering although it is admitted that no distinction can be made between developing and atretic follicles and that only follicles over a certain size can be palpated. Choudary, Gier & Marion (1968) showed however that no cyclic changes occur in follicles up to 5 mm in diameter which suggested that follicular growth, rather than being cyclic, proceeded from one size of follicle to another independant of specific days of the cycle.

Furthermore, it is clear from this investigation that the nutritional level exerts such an overwhelming effect on ovarian activity, that any observations on follicular development during the cycle, should take the level of feeding into account.

To this has to be added important individual differences that were indicated earlier on.

3.1.7 Quiescent estrus

Quiescent estrus was recorded when ovarian changes and vaginal discharge were observed as in the case of estrus but unaccompanied by overt behavioural changes or receptivity to other females.

During the heifer stage, quiescent estrus occurred in 4,6 per cent of the 259 estrus periods studied. There did not appear to be any individual susceptibility. It was recorded once in each of 5 heifers, twice each in two heifers and in one heifer only on three occasions.

As adults, quiescent estrus was recorded in 8,9 per cent of 374 cycles studied. The incidence was the same for the HIGH and the LOW group.

The first two estrus periods after the onset of puberty in the heifers and the first two estrus periods after calving, were characterized by a higher incidence of quiescent estrus than subsequent cycles.

The incidence of ovulatory failure at quiescent estrus periods was no higher than at normal estrus periods and the impression was gained that ovarian activity was re-established after calving sooner than estrus behaviour, and also sooner than normal cycle length.

According to Cole & Cupps (1969) quoting Robinson, progesterone sensitization is needed before the full response to estrogen is manifested, which explains this phenomenon as well as a possible role for the corpus luteum in cycle length.

Callaghan, Erb, Surve & Randall (1971) on the other hand, postulate that re-establishment of cyclic activity after calving depends on initial over-stimulation by FSH and consequent high estrogen production, which in addition to inhibiting FSH temporarily, causes morphological alteration in the follicles and alters steroid synthesis to favour progestin instead of estrogen which initiates further release of FSH. They recorded levels of plasma progesterone in cows showing anovulatory estrus or failure to form a corpus luteum after mild estrus, to be comparable to levels observed in cows with palpable corpora lutea.

Graves and his coworkers (1968) reported on the role of suckling in quiescent estrus. They recorded quiescent estrus at first ovulations after calving of

42,4 per cent in non suckled and 70,6 per cent in suckled cows. Both the interval since calving and suckling increased total pituitary FSH content at 15 days after calving and suckling increased follicular fluid weight on day 3 after calving.

Other observers reported a higher incidence of quiescent estrus than recorded in this study.

Kidder, Barrett & Casida (1952) recorded a 27,3 per cent incidence of quiescent estrus in a large herd over all the cycles with 44,3 per cent during the first 60 days after calving. Morrow (1969) in a study of normal cows and cows that suffered from post parturient complications recorded a 77,1 per cent frequency of quiescent heat at first estrus, 54,4 per cent at second estrus and 35,8 per cent at third estrus after calving without any difference between normal and abnormal cows.

Salisbury & Vandemark (1961) again, quote recorded incidence of silent estrus to vary from 18,8 to 27,3 per cent and they provide evidence that conception is just as high when cows are inseminated at silent estrus than at normal estrus.

After extensive investigations on post partum ovarian activity in cows, Casida (1968) reported that a common observation during the post partum interval has been that follicles may develop to mature size or even larger and then become atretic or regress, followed by the development of further follicles, one of which eventually ovulates without inducing estrus in 46,5 per cent of calving intervals, or it induces estrus without ensuing ovulation in 10,5 per cent of calving intervals.

It is clear from these observations that quiescent estrus is most frequent immediately after calving and during the first few cycles after the onset of puberty in heifers. Considerable variation has been recorded in the incidence of quiescent estrus at other times in cattle.

This investigation confirms the gradual establishment of hormonal activity, after the onset of puberty and again after calving, for cyclic activity and for estrus.

No difference in the incidence of quiescent estrus could be established at either stage between the HIGH and LOW feeding groups.

The question arises, however, whether, particularly in large herds, missed estrus periods, as well as short estrus periods, play a role in the high incidence of anestrus ovulations and the variation in results reported by different observers.

3.1.8 Conception in relation to ovulatory performance

In the course of this investigation a total of 134 inseminations were carried out at three different breeding periods.

The number of inseminations received by each animal together with her respective ovulatory record at the time of insemination is expressed by the following symbols and is summarized in Tables 4(a), 4(b) and 4(c) for the three breeding periods.

+	normal ovulation
0	anovulation
θ	delayed ovulation

From Table 4(a) it is clear that the high incidence of functional disturbances in the ovaries of the heifers selected to Group I was reflected in their poor conception rate on insemination as compared to Group II and Group III.

It is further quite obvious from Tables 4(a), 4(b) and 4(c) that the high nutritional level at each breeding period, has been responsible for a lowered conception rate. In the HIGH group after the second calving according to Table 4(c), reduced fertility reached such proportions that, for purposes of production under practical farming conditions, this group would obviously have been economically disastrous.

The factors responsible for the failure of the HIGH group after the second calving, will be elaborated on at a later stage.

The fertilization rate in respect of ovarian activity and ovulatory failure at estrus can be summarized as follows:

Normal ovulations (+) followed by conception	59 times (44 %)
Normal ovulations without conception	33 times (24,6%)
Delayed ovulation (θ) followed by conception	9 times (6,7%)
Delayed ovulation without conception	16 times (12,0%)
Anovulation	17 times (12,7%)

TABLE 4 — The number of inseminations received per animal and record of ovulations at three breeding periods: (a) as heifers (b) after first calving and (c) after second calving

*Denotes not in calf (+ - normal ovulation; 0 - anovulation; θ - delayed ovulation)

4(a) As Heifers

Group I (Low fertility)			Group II (Constant level of feeding)			Group III (Reduced level of feeding)		
Number of Heifer	Number of Inseminations	Ovulatory record	Number of Heifer	Number of Inseminations	Ovulatory record	Number of Heifer	Number of Inseminations	Ovulatory record
23*	1	0	8	1	θ	6	2	θ θ
22	5	+ + +	15	1	+	4	1	+
13	4	0 θ 0 θ	25	2	+ +	2	1	+
11	5	θ + 0 θ	21	1	+	5	1	θ
20	1	+	10	3	+ 0 θ	14	1	+
12	3	+ θ +	17	3	+ 0 +	3	2	+ +
31*	9	+ + 0 + + + + + +	19*	2	θ 0	7	1	+
24	2	+ θ	32	1	+	26	1	+
29	1	+	9	2	θ +	1	2	+ +
30	1	+	18	1	θ	16	2	θ +
			28	2	0 θ	27	1	+
Total	3,2 insemination per animal. Two not in calf.		1,7 insemination per animal. One not in calf.			1,4 insemination per animal. All in calf.		

4(b) – After first calving

LOW level of feeding			HIGH level of feeding		
No of cow	Number of insemination	Ovulatory record	No of cow	Number of insemination	Ovulatory record
1	2	+ +	2	1	+
3	1	+	5	2	+ +
4	1	+	7	2	+ +
6	2	+ +	9	1	+
8	1	+	12*	4	0 Θ + +
10	2	Θ +	14*	4	Θ 0 + +
13	1	+	17	2	+ +
16	1	+	18	2	+ +
20	2	+ +	24	3	0 Θ +
21	1	+	25	1	+
27	1	+	30	2	Θ +
29	1	+	32	2	0 +
Total 1,3 inseminations per animal. All in calf.			2,2 inseminations per animal. Two not in calf.		

4(c) – After second calving

LOW level of feeding			HIGH level of feeding		
No of cow	Number of insemination	Ovulatory record	No of cow	Number of insemination	Ovulatory record
1	1	+	2	–	Dead
3	1	+	5	–	Anestrus
4	1	+	7	2	0 +
6	1	+	9	1	+
8	2	0 +	12*	5	+ 0 + + +
10	1	+	14	–	Anestrus
13	1	+	17	–	Dead
16	1	+	18	–	Anestrus
20	1	+	24*	2	0 ⊖
21	2	+ +	25*	1	+
27	1	+	30	–	Anestrus
29	1	+	32	1	+
Total 1,2 inseminations per animal. All in calf.			12 inseminations, four in calf.		

Semen of the same bull of known high fertility was used for all inseminations.

The incidence of normal ovulations that failed to conceive, could be explained along several pathways.

The timing of the ovas entry into the uterus depends on the level of estrogen and this is critical because premature entry leads to expulsion from the uterus, and an extended stay of the blastocyst in the fallopian tube, leads to degenerative changes (Adams, 1967). Adams (1967) also quotes results reported by Dickman & Noyce and concludes that survival chance in the uterus not only depends on the absolute stage of maturation of the ova and uterus, but also upon a particular relationship between the stages of development of the ova and uterus.

Chang (1967) reports further that the capacitation of sperm in the uterus is a reaction that depends on uterine components which are all controlled by female hormones, in particular estrogens and progestogens and the relationship between them. (Emmens (1969)).

Unripe or aged ova or an endometrium that is hostile to sperm fertility or survival of the blastocyst therefore seems to depend to a considerable extent on hormonal balance which in the light of previous experience with the present females is delicate and even precarious.

The paradoxical incidence of conception following ovulatory failure affords information on the survival time of sperm in the female tract.

In the nine cases quoted, sperm was found to survive for the following minimum periods from insemination until ovulation was recorded:

24 hours	–	5 times
36 hours	–	once
48 hours	–	twice
72 hours	–	once

Salisbury & Vandemark (1961) quote various reports and give the survival time as between 22 and a maximum of 56 hours with sperm living longer in the uterine fluids of cows at estrus than during the luteal phase.

Bishop (1960) records sperm survival time in the female tract as between 22 and 50 hours while Van Rensburg & De Vos (1962), encountered no cases of conception in their investigations when ovulation was delayed for more than 24 hours after insemination. In their work inseminations were carried out at daily intervals until ovulation had occurred and they concluded that the cause of failure of fertilization was the viability of sperm and not ova after delayed ovulation.

Fugo & Butcher (1966) and Butcher, Blue & Fugo (1969) working on rats, demonstrated beyond doubt that delayed ovulation leads to overripeness of ova which results in embryonic death and developmental defects. It further decreased implantation through changes in the uterine environment, which is in agreement with the reports of Adams (1967), Chang (1967) and Emmens (1969) quoted above.

From Groups II and III in Table 4(a) and from the HIGH and LOW feeding groups in Tables 4(b) and 4(c) it can be seen that 14 inseminations were carried out at estrus periods when normal ovulations (+) were recorded but when conception failed in animals on a high feeding level, against only 6 cases in animals on a low feeding level.

In Group I in Table 4(a) no less than 13 inseminations failed with normal ovulations (+) at estrus against 6 inseminations with ovulatory failure at estrus.

Although numbers are small to draw final conclusions, it is obvious that the conception rate of animals on the higher feeding level was reduced. Failure of conception in spite of normal ovulation at estrus was responsible for this, rather than failure of conception because of ovulatory failure.

The question arises whether endocrine imbalances that were harmful to fertilization were induced by the high feeding level. One could speculate, according to Figures 4(a) and 4(b), that differences in the level of estrogens produced by the ovaries, are feasible.

The remarkable response of the uterus to estrogens is well recorded and in addition to some effects of estrogen on fertilization that have already been mentioned. Villee (1961) and Emmens (1969) provide us with a full account of the effect of estrogens on the uterus and conclude on the influence of smaller follicles on the ripening follicle that "the favoured follicle truly stands upon the shoulders of its contemporaries".

The effect of a high nutritional level and particularly a high energy level on embryonic survival rate is well recorded in sheep and swine (Ahmed S. El-Sheikh, C.V. Hulet, A.L. Pope & L.E. Casida 1955, J.W. Gossett & A.M. Sorenson (Jnr) 1959, Zimmerman, Spies, Self & Casida 1960, Sorenson, Thomas & Gossett 1961, and Bellows, Pope Chapman, & Casida 1963). Ovulation rate, follicular size and numbers of follicles as well as fertility rate are increased but embryonic survival rate is significantly decreased by high feeding levels. The dangers of overfeeding are further illustrated by the work of Arnett & Totusek (1963) who recorded 1,70 services per conception in heifers on high energy diet compared to 1,53 for low energy diet in twin heifers in drylot for three lactations.

It was clearly demonstrated in this study that delayed ovulation leads to failure of fertilization. Out of 25 cases of delayed ovulation at insemination, 16 failed to conceive. In the other 9 cases, semen survived in the female tract rather longer than was expected according to previously published results. This considerable difference might have been effected by improved techniques of semen evaluation and storage employed in this study compared to procedures at the time of the previous reports.

3.1.9 Body mass

Average body mass in kg as recorded for the two groups at monthly intervals, are illustrated in Figure 5 from after the first calving for a period of 25 months until the end of the trial.

For the first three months in Figure 5, both groups were kept on the same level of feeding as explained, and during this period the difference in average body mass was small and remained unchanged. After the groups were seperated at the fourth month and put on the high and the low feeding levels, a marked difference developed in average body mass between the two groups. This difference was maintained throughout the duration of the trial, averaging 151 kg per animal during the final 15 months.

It is further evident from Figure 5 that the post calving drop in average body mass was of greater intensity in the HIGH group (89,8 kg or 12,9%) than in the LOW group (59,9 kg or 11,1%) particularly since 2 cows in the HIGH group failed to conceive and actually gained in mass during the period concerned.

FIGURE 5 — Monthly average body mass of cows on a high (H) and a low (L) level of feeding from July 1969 to July 1971

This illustrates the severity of the effect of post calving stress in the HIGH group since the relative birth mass of calves from the LOW group was in fact higher (6,4%) than that of calves from the HIGH group (5,7%).

3.1.10 Temperature, heart and respiratory rates

The average temperatures, respiratory and heart rates taken at various intervals during 1968 and until 1971 as indicated, are illustrated in Figure 6.

Data on the LOW nutritional level in 1968 were collected from those heifers that were selected to Group III and put on a reduced level of feeding six weeks before insemination.

Data on the HIGH nutritional level in 1968 were collected from those heifers that were selected to Groups II and III and kept on an unaltered feeding level during inseminations.

Fluctuations in heart and respiratory rates within groups between the various dates at which recordings were taken according to Figure 6, corresponded to fluctuations in the ambient temperature as supplied by the weather bureau for the specific dates. These fluctuations evidently represent the normal seasonal variations.

On 1/8 and 15/9 during 1968 and on 25/7, 15/8 and 20/9 during 1969, heart and respiratory rates were equal, respectively, for both groups. This was during periods when all animals were on the same nutritional level.

Of particular importance according to Figure 6, is the fact that the high feeding level accelerated the heart as well as the respiratory rates considerably regardless of variations in ambient temperature, while body temperatures remained remarkably constant. The averages over this 3-year period can be summarized as follows:

	Same level of feeding		Different levels of feeding	
	HIGH group	LOW group	HIGH group	LOW group
Heart rate	73,8	74,4	79,2	66,1
Respiratory rate	36,2	35,8	47,5	36,3
Body temperature	39,15	39,2	38,9	38,9

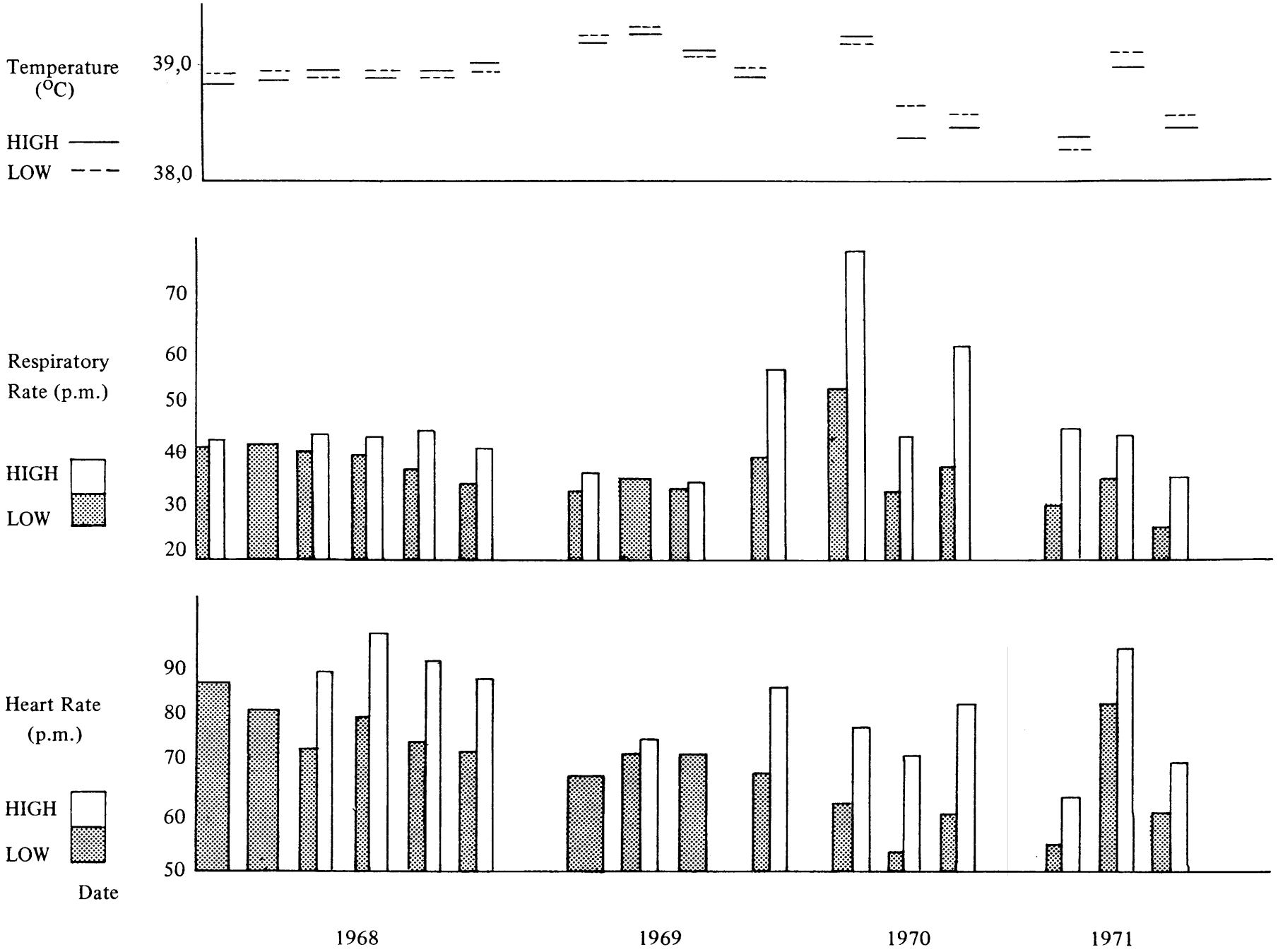


FIGURE 6 – Temperature respiratory and heart rates of cows on HIGH and LOW nutritional levels

It is clear from this summary that there was, over the full 3 year period, an average increase of 20% in respiratory rate and 31% in heart rate respectively in the animals on *ad libitum* feeding compared to their counterparts on a controlled feeding level.

Metabolic rate, and therefore heart and respiratory frequency, is a function of body mass, the metabolic rate being lower per unit of mass in bodies of large than in bodies of small size. (Hafez, 1968). If body mass is therefore taken into account in the two groups employed in this investigation, it is clear that the larger size or mass of the animals in the HIGH group, augments the differences recorded in heart and respiratory frequencies; particularly since the difference in average body mass is made up essentially of adipose tissue which in cattle has a maintenance cost comparable to that of the body as a whole (Blaxter, 1962).

Berman (1967), working on thermal stress in bovines, reported that cattle on *ad lib* feeding displayed respiratory frequencies 44% higher and a ventilation rate 25% higher in summer than controls on a restricted level of feeding. The feeding level also had a significant effect on respiratory vaporization which was 40,9% higher in spring and 36% higher in summer in cattle on the higher feeding level. In the low feeding group, vaporization reached diurnal peak much earlier in summer than the higher feeding group where it continued to rize during the later hours even though air temperature remained the same.

The long term effects of cardiac and respiratory acceleration, have never been reported on in the bovine, neither is any direct evidence available on fermentation heat *per se* as a stressor in cattle. It may be argued however, that fermentation or digestive heat production is analogous to environmental heat in the ruminant, which is well recorded as a limiting factor in the production and reproduction in bovines. (Ulberg, 1958; MacFarlane, Pennycuik, Yeates & Thrift, 1965; Bonsma & Louw, 1966; Venter, Bonsma & Skinner, 1973.) Reproduction in particular is severely affected. Body temperature of 0,5 to 1°C above normal in the cow at the time of insemination, can affect the mortality of the embryo 30 to 40 days later*.

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* Embryonic mortality related to temperature. Agricultural Report No. 116. 8 Febr. 1974. Embassy of South Africa, Washington D.C.

Bonsma & Louw (1966) pose the question whether two mechanisms are involved in the effect of high environmental temperatures on bovine reproduction vis. reduced fertility due to malnutrition and resultant degeneration as well as the direct effects of hyperthermia upon the reproductive process. According to Ulberg (1958) the ova of females under constant high temperatures may be affected prior to fertilization whereas less severe or variable temperatures bring about a change in uterine environment and death of the embryo prior to implantation.

Van Rensburg & De Vos (1960) reported a distinct seasonal fluctuation in the incidence of ovulatory failure in bovines but they failed to seperate fluctuations in nutritional level from changes in environmental temperatures.

3.1.11 Laminitis and arthritis

The laminitis/arthritis syndrome presented one of the most serious problems in the HIGH group, while no cases were recorded in the LOW group. Lameness presented such a severe strain indeed that, without any of the other complications in the HIGH group, economic production would have been rendered impossible only because of problems with legs and feet in the HIGH group.

During the heifer stage no laminitis and no lameness from any other cause was recorded. After calving with the establishment of the HIGH and LOW feeding groups however, a gradual growing out of hoofs in the HIGH group, and particularly in the hind feet, were recorded.

Not a single case of acute laminitis was recorded during this period however until after the second calving.

Growing out of hoofs occurred in a spiral fashion in the toes so that the weight of the animal was transferred to the heels. The gait was painful and walking ability was seriously impaired. Clipping of the hoofs had to be done regularly which relieved the pain on walking considerably.

The post parturient period brought about a very serious deterioration in lameness in the HIGH group whilst still no cases occurred in the LOW group.

Acute laminitis developed during the puerperium and it was characterized by severe pain and heat with swelling and congestion of the skin of the coronet,

which was obvious in unpigmented areas. Laminitis was recorded either independently or in association with metritis, which was common in the HIGH group during this period.

Arthritis, characterized by a painful swelling of the joint with distension of the joint capsule, was further recorded during the post parturient period either independently or in association with laminitis.

The distribution of laminitis between hind and front feet, as well as the incidence of arthritis is apparent from the records of individual animals which are now briefly presented.

Number 2. Carpal, tarsal and stifle joints on all four legs swollen and painful with swelling of the coronet and grown out hoofs on all four feet.

Number 5. Hoofs on both hind feet grown out, particularly the outer hoof, but pain on walking mild and sometimes absent.

Number 12. Both hind feet severely grown out and both tarsal joints swollen. Front feet only slightly grown out and no symptom of arthritis in the front legs.

Number 14. Hind feet both grown out and tarsal joints swollen. Gait severely painful. Front legs and feet normal.

Number 18. Only outer back hoofs grown out and only right stifle joint swollen and severely painful. Both carpal joints swollen and severely painful but both front feet normal.

Number 24. Outer back hoofs both grown out and right hind fetlock swollen and painful. Front feet normal.

Number 25. Both hind feet grown out and painful and both hind fetlocks swollen and painful. Front hoofs both normal but both fetlocks in front swollen and severely painful.

Number 7. Back feet both only slightly grown out but she walks with ease and without any sign of pain. All joints normal.

Number 17. Both hind feet severely grown out and painful with swollen and painful hock joints and right stifle.

Number 9. All four feet and all joints normal and she walks without any discomfort.

Number 30. Both hind feet severely grown out and she walks with considerable discomfort.

Number 32. Both hind legs severely painful. Hind feet are swollen and inflamed with the hoofs severely grown out and twisted. Right hind fetlock is swollen and hardly any weight is put on the right hind leg, while the fetlock on the left hind legs appears to be normal but buckles forward.

In the LOW group no symptom whatsoever of laminitis, arthritis, growing out of hoofs or lameness was recorded during the entire period.

It is clear from the above records that only 2 out of the 12 cows in the HIGH group escaped laminitis and/or arthritis. The post partum period brought about serious aggravation in these symptoms.

The two cows in the HIGH group that failed to conceive however, likewise suffered severely from arthritis and laminitis. Cows numbers 7 and 9 on the other hand appeared to have had an inherent resistance against laminitis or arthritis in spite of the fact that they even suffered from post parturient metritis.

It is obvious too, that hind feet were more susceptible than front feet and in two cows, only the outer back hoofs were affected while the inner hoof remained quite normal.

The reason for this is not clear. One is tempted to argue that hygienic considerations or soaking of the hoofs might play a role, the hind feet being soiled more often than the front feet.

In the two cows where only the outer hoofs were affected one might suspect that conformational features might have induced a predisposition to laminitis, but this was not obvious from an inspection of the feet and legs.

It is further reasonable to argue that secondary arthritis follows on laminitis which explains the incidence of arthritis in the fetlocks, carpus, tarsus and stifle joint and that bacterial infection might have played a role.

It is however along these lines, impossible to explain the incidence of arthritis in the fetlocks and carpi that was unaccompanied by laminitis, neither is it clear how carpal and tarsal joints became affected without the fetlocks developing symptoms.

Taking the complete abscence of laminitis and arthritis in the LOW group into account, it becomes clear that hygienic considerations could only have played a very minor role.

The odd distribution of arthritis and laminitis, can hardly be explained along the lines that these conditions are of a secondary nature. With the incidence recorded in this investigation, it is highly unlikely that the arthritis was secondary to laminitis and that hygiene played any role at all.

The pain, heat and swelling that accompanied laminitis was commonly recorded and was particularly distinct in the skin above the coronet whenever it was unpigmented. Maceration of the soft horny tissue of the bulb of the hoof and interdigital dermatitis were sometimes recorded and were strongly reminiscent of "footrot", (interdigital necrobacillosis).

For practical reasons Fusiforums necrophorus infection could naturally not be eliminated beyond any doubt in all the cases recorded as laminitis. It was endeavoured however to eliminate infectious pododermatitis by the systemic administration of wide spectrum antibiotics. Corticosteroids were frequently used as well. In addition, cows were made to walk through a copper sulphate footbath daily.

In the event therefore of pododermatitis having been erroneously recorded as laminitis, it should be viewed as a condition that failed to respond to regular intensive treatment and prophylactic measures instituted at a very early stage against the former conditions.

In any event, typical interdigital ulceration and necrosis in the initial stages of lameness, followed by sepsis of the phalanges and abscessation of the coronet in long standing cases, which are all typical symptoms and complications of footrot, were never recorded.

Footrot furthermore usually affects one limb only and laminitis two or more, and this was regularly recorded. Affected cows walked with extreme caution with a painful gait and an arched back. In long standing cases cows were seen to stand with their hind legs well back without favouring any leg in particular.

Hygienic conditions were of a high standard throughout and exactly identical for the two groups.

Laminitis in cattle is well recorded in literature. The definition is a diffuse acute or chronic aseptic pododermatitis usually affecting several digits (Greenough, MacCallum & Weaver 1972). Cases recorded in this study fit this definition precisely.

Toussant Raven & Cornelisse (1971) presented a review of the inflammatory conditions of the interdigital skin in cattle. They report two infectious conditions of the bovine foot, one an inflammation of the subcutaneous tissue in the interdigital area, and the other an inflammation of the interdigital skin itself. Both conditions lead to granulating proliferation of the interdigital skin and abnormal horn formation in the bulb region with "hoof" or "sole" ulcer formation in the area. They report however, that neither condition produces excessive growing out of the hoofs in the wall or solar areas.

In this study, congestion of the skin and subcutus was most evident above the coronet but was unaccompanied by the degenerative granulation and proliferation mentioned above. Hoof or sole ulcers were not recorded. The essence of the problem in this study was elongation of the wall of the hoof anteriorly with inflammatory changes to the laminae and to the joints and bony tissues as described.

Lesions in the soft tissues of the joints and in the laminae were reflected most prominently as bony deposits on the underlying tissues.

Exostosis of varying sizes had developed on the pedal bones and phalanges as well as on the carpal and tarsal bones following arthritis in the carpal and tarsal joints. In two cows deposits of a calcareous appearance had developed on the proximal part of the metacarpus as a sequel to carpal arthritis.

Fritsch (1966) in a review of the aetiology of hoof ulceration in cattle regard the abscence of a soft mattress or surface as the main cause of contusion and eventual ulceration, perforation and necrosis.

The present study does not support this view. A strip of thick bedding was available all along the feeding troughs where cows were tied up while feeding. In between however, no cows were noticed to give preference to standing on bedding instead of the bare concrete portion of the paddock however painful their feet might have been.

Fritsch also records the normal moisture content of the cutis of the wall and sole as 15 to 20 per cent. Additional take-up of water on prolonged standing in water or dung was 5 to 6 per cent and further absorption was not possible. No decomposition or liquefaction of the horn, or abnormal softness which eventually produced tenderness, or inflammation was however recorded and he concluded that damp bedding was innocuous to cattle.

Summarily then, animals on the high feeding level not only suffered from severe laminitis which is well recorded in literature, but also from aseptic arthritis which was not necessarily associated with laminitis. No reports are available to explain the aetiology of this condition in bovines on a high feeding level.

The severe susceptibility of certain individual bovines to both arthritis and laminitis and the complete resistance of others under the same conditions, is a most essential subject for further investigation.

The incidence of laminitis is known to be high in fattening cattle (McLean, 1966) and to be encountered in dairy cattle particularly in association with metritis during the puerperium and also with concentrate feeding (Nilsson 1963, MacLean 1965) while de Boom, Adelaar and Terblanche (1968) reported on an inherited susceptibility of Jersey cattle to laminitis.

Brown, Roussel & Stallcup (1967a) postulated a possible genetic basis to the disease which in their investigation was recorded in related but not in unrelated Hereford bulls.

It is agreed that laminitis is more common and more severe in the hind feet and Nillson (1963) reported that in his studies, Frieslands were more commonly affected in the hind feet, while Swedish Red and White cattle were more often affected in the fore feet.

Nillson also provides evidence that histamine is involved in the pathogenesis of the disease in cattle and he even indicates that histamine might be involved in the retention of the fetal membranes. In his studies however, the examination of placental secretions for histamine, has not given any definite evidence that histaminosis arises from the content of the uterus with retained fetal membranes. He points out that large quantities of histamine are retained in the wall of the uterus in connection with parturition and he concludes that in the so called parturition laminitis cases, retained fatal membranes and laminitis might be different expressions for a common allergic syndrome.

In metritis, histaminosis might be brought about by the breakdown and destruction of tissue by bacterial action and Nillsson (1963) quotes the incidence of metastatic laminitis in horses combined with infectious processes other than metritis e.g. sternal abscess, purulent pericarditis, purulent valvular endocarditis etc.

One might believe that laminitis could be brought on in connection with an allergy towards the pus in the lesion. An alternative possibility is that metastasis might be associated with the distribution of primary infection from sites to the hooves. In the latter event, the definition of laminitis as an aseptic inflammation, does not fit in.

Histamine and tyramine have been identified as toxic constituents in the ruminal ingesta of experimentally overfed sheep by Davis, Neal & Dougherty (1955) and in overfed cattle by Ahrens (1967).

MacLean (1970c) studied the haematology of bovine laminitis and recorded significantly higher levels of histamine in dairy cows with laminitis, with a tendency for affected cows to show increased packed cell volume, haemoglobin levels as well as S.G.O.T. elevation in cows with chronic laminitis.

Symptoms reported by Davis, Neal & Dougherty (1955) and by Ahrens (1967) were found to be directly correlated with the level of histamine and as the acidity of the rumen became lower than pH5, histamine formation increased.

Fuquay, Kesler & Zarkower (1969) on the other hand, failed to relate high levels of histamine in the rumen of Frieslands to a reduction in rumen pH. They reported that animals preconditioned with low levels of concentrates before

changing to a high concentrate diet at parturition, showed higher histamine concentration in the rumen than animals going from an all hay diet to a high concentrate diet or than animals maintained on a low concentrate diet during the post parturient period.

Furthermore, the incidence of lameness related to treatment in their animals could not be explained entirely in terms of circulating histamine levels. They concluded that undetermined factors must be involved since no cows in groups with the highest histamine levels were adversely affected.

These observations together with the records of laminitis in this study, can only be explained in terms of the distinct individual variations observed within the HIGH group employed. These variations should be investigated in relation to possible genetic differences in the pathways of histamine metabolism.

MacLean (1971) examined histological changes in cases of laminitis and reported that vascular changes are primary and are associated with an increase in blood flow and capillary dilatation, stasis and stagnant hypoxia which result in permanent damage to the laminae unless the process is quickly reversed. The onychogenic substance disappears and the stratum germinativum becomes disorientated with altered nuclear appearance.

MacLean (1970a) also recorded rarefication of the pedal bone in laminitis and enlargement of vascular channels of the foot. A higher moisture content and a reduction in sulphur containing amino acids in affected hooves were further reported by MacLean (1970b).

The incidence of arthritis and in particular the cases recorded here, where there was no prior record of laminitis, presents a problem in aetiology which requires further investigation.

Fetlock, carpal and tarsal joints were clinically affected. Animals walked with difficulty with one or more joints showing acute or subacute inflammation as judged by the amount of pain, heat and swelling in the joint.

At slaughtering synovial fluid appeared to be increased in amount and slightly turbid. Articular cartilages appeared normal but petechial haemorrhages were sometimes present on the joint capsule with gelatinous infiltration of the surrounding

tissue. Frequently however, a swollen joint appeared quite normal on visual inspection at slaughtering apart from possible increase in the amount of synovial fluid.

Microscopic examination of tissues in affected joints might have revealed more lesions.

It was assumed that the arthritis that was recorded in this study was of a degenerative type mainly because abscessation and suppuration which are regular features of infectious arthritis, were never recorded, even in the long standing cases or at slaughtering.

It is further highly unlikely and contrary to clinical experience that arthritis of infectious origin would occur adjacent to laminitis of non-infectious origin as discussed earlier on.

Degenerative arthritis is well recorded in bovines and an account of the incidence and aetiology as reported by various observers, is presented by Greenough, MacCallum & Weaver (1972).

They report that most cases of coxitis, which were not recorded during this investigation, is non-infectious and of a degenerative nature, while gonitis, which was attributed to injury or mechanical strain, remained of obscure origin apart from its common infectious nature.

Aseptic tarsitis according to their summary occurs undoubtedly in cattle and can be regarded as a manifestation of the osteo-arthritic syndrome, equivalent to equine "bone spavin". Abnormal conformation and excessive stress, (which is not specified) are mentioned as possible aetiological factors while in one report it was linked in well-fed bulls with a nutritional aetiology.

Weaver (1972) reported that detailed pathological examination of joints of fattening cattle has revealed that abnormalities are present in many joints which may not necessarily lead to lameness but to unthriftiness and which bears resemblance to similar problems in pigs.

Jubb & Kennedy (1970) admit the possibility that non-infectious arthritis may occur in animals and they suggest that it is of the nature of a hypersensitivity,

with arthiritis and tendo-vaginitis occurring simultaneously, each being primarily a synovitis.

Summarily therefore, the direct mechanism responsible for arthritis in this study and from literature, is nebulous. In the present study, its incidence in the HIGH group and its complete abscence in the LOW group point to a nutritional aetiology in which individual animals differ considerably in susceptibility.

Closer scrutiny and more purposeful records will undoubtedly reveal further evidence in support of the impression gained in this investigation that laminitis and degenerative arthritis play a far more important role in bovines on a high level of nutrition than generally realized.

Laminitis is well recorded in literature but no reports are available on the incidence of aseptic arthritis as a herd problem in bovines. The aetiology of individual cases that are on record is unexplained. The severe susceptibility of certain individual bovines to arthritis and to laminitis and the complete resistance of others of the same type and under the same conditions, is a most essential subject for further investigation.

3.1.12 Milk production

Although the purpose of this investigation was not to study milk production on different feeding levels, the respective amounts of milk produced by the two groups and particularly the estimated financial returns between the two treatment groups served most dramatically to accentuate the fallacy of *ad libitum* or over-feeding of the dairy cow.

Feeding treatments were commenced with during the second half of the first lactation and production for this period was disregarded in this comparison.

The following milk production was recorded for each group during the first 120 days of the second lactation:

No. of cow	Total milk production (kg)	Average daily production (kg)	Duration of lactation (days)
LOW Group			
1	1410	11,7	120
3	907	7,6	120
4	1168	9,7	120
6	782	6,5	120
8	864	7,2	120
10	1082	9,0	120
13	805	6,7	120
16	849	7,1	120
20	1353	11,3	120
21	1257	10,5	120
27	1100	9,2	120
29	1476	12,3	120
Average	1088	9,07	
SD	230	1,97	
HIGH Group			
2	188	7,8	24
5	1305	10,9	120
7	1674	13,9	120
9	1498	12,5	120
17	142	8,9	16,0
18	250	7,6	33,0
24	1526	12,7	120
25	1318	11,0	120
32	428	4,7	92
31	19,1	1,6	12
14	0	0	0
12	0	0	0
Average	696*	7,63**	
SD	665	4,78	

* $P < 0,05$ ** $P < 0,01$

A significant difference ($P < 0,05$) and a highly significant difference ($P < 0,01$) are evident in total milk production as well as in average daily production respectively in favour of the LOW group. Admittedly production records were profoundly affected by the severe erosion of cows in the HIGH group. This however is the essence of this investigation. It has been motivated that conditions and

feeding regimes employed in this study might well pertain to certain present day farming and breeding practices and it is suggested that the same dismal record of milk production is by no means unlikely under similar conditions.

It is further clear from the above records that 7 out of the 12 cows in the HIGH group failed to complete 120 days of their second lactation. Cows numbers 2 and 17 died from metabolic disease as will be indicated further on. Number 18, which was dry after only 33 days, suffered severely from laminitis and arthritis with degenerative changes in her kidneys and endocarditis, while cow 32 was dry after 92 days with severe laminitis. Cow 31 suffered a spontaneous rupture of the uterus at calving. Her calf was delivered by caesarian operation which was uncomplicated but she produced almost no milk afterwards. Cows numbers 12 and 14 failed to conceive.

The production of the remaining 5 cows in the HIGH group together with their feed intake as against that of the LOW group, was as follows:

	HIGH group	**LOW group**
	(120 days lactation completed)	
Hay – average daily intake in kg	2,49	8,42
Dairy concentrate – average daily intake in kg	12,70	4,08
Average daily milk production (kg)	27,2	20,0

It is quite clear that the difference in milk production in the group on *ad libitum* feeding level was not justified by the higher level of feed consumption. If the heavy erosion of cows on the higher level of feeding is added, the dangers of *ad libitum* feeding in dairy cows hardly need further emphasis.

3.1.13 The process of parturition

The behaviour of individual animals differed markedly at parturition within the treatment groups. Some cows exhibited intense labour or straining at birth, stopped feeding and went down at an early stage after the onset of labour or even before cervical dilatation was complete.

It was a distinct feature in some of the cows to start straining before completion of cervical dilatation. This resulted in rupturing of the fetal membranes at

a stage even before the fetus was intrapelvic or the cervical opening large enough for its passage. It was strongly suspected that this rather premature onset of labour, resulted in the early detachment of the placenta, which led to fetal suffocation at a much earlier stage in some cows than others.

In other cows again, cervical dilatation was complete and wide enough for easy passage of the fetus while the cow would continue feeding and even ruminating without showing any sign of straining or the onset of labour, often with the legs of the fetus and fetal membranes presented in the pelvic outlet.

Although the myometrium as well as the abdominal muscles contribute to the expulsion of the fetus, it was not possible with internal examination during labour to distinguish between the respective contractions of these muscles. Abdominal straining at palpation is of such severity that contractions of the myometrium are completely overshadowed.

There is no direct method to establish the exact stage at which assistance should be rendered so as not to deliver a calf that would have been born naturally on the one hand or to sacrifice a calf that might have been saved on the other hand.

Cows were assisted when on the strength of previous experience, it was suspected that natural birth was unlikely or that the survival of the fetus would unnecessarily be endangered.

3.1.13.1 The first calving

With their first calving as heifers, 3 out of 27 calves that were born had to be delivered by traction. Placentae were retained in two cases and had to be removed manually with the usual after treatment. A further three heifers required treatment for post parturient metritis.

Uterine involution and the re-establishment of cyclic activity were uneventful and all the heifers that calved retained their full fertility.

3.1.13.2 The second calving

At the time of the second calving, the effect of the high level of feeding was fully established. Parturition was severly complicated and characterized by pro-

longed periods of labour while the post parturient recovery in the HIGH group was complicated by a delay in the completion of uterine involution and a high incidence of metritis.

The approach to the investigation of the performance of the bovine during the post partum period in this study, will be briefly outlined first. It is incompletely recorded in literature or text books on the subject and the data to be presented on the performance of individual cows, is to be interpreted according to this approach.

It is based on conceptions gained over many years in the treatment of post calving complications in cattle and it was clearly confirmed by the close scrutiny that was possible during this investigation.

Valuable information can be obtained by rectal palpation on the state of the uterus in the cow and by mild massaging of the uterus by rubbing it from in front of the pelvic inlet backwards. The outlines and size of both the pregnant and non pregnant horns of the uterus can be determined as well as the tonus of the uterine wall.

The myometrium reacts to palpation and the longitudinal muscular layer contracts and can be felt as a series of fine, but firm corrugations extending over the pelvic brim.

These fine ridges are absent when the uterus lacks tonus and the firmness of the uterine wall gives way to flabbiness. With very few exceptions, a delay in uterine involution can be expected during which the lochia putrefies. This forms an excellent environment for bacterial proliferation and septicaemia.

With massaging of the uterus the presence of fluid, and to a certain extent the amount of fluid, can be ascertained, particularly when there is a lack of uterine tonus. It is invariably possible to obtain some of the fluid by massaging it out and the physical properties of this fluid is most important in the clinical evaluation of the state of the endometrium.

The normal uterine contents or mucus after calving is odourless, quite viscous and often tainted with blood or blood pigment. Liquefaction of the mucus is invariably associated with lack of uterine tonus and is frequently followed by putre-

faction, purulence and an offensive odour and septicaemia. This never happens when the tonus of the uterine wall and the viscosity of the mucus are maintained.

In the abscence of any specific venereal infection and as a stage in uterine involution, the presence of thick yellowish custard-like pus, with or without admixture with mucus or blood, signifies an end result of putrefaction of lochia and a stage at which secondary or general symptoms have usually cleared up.

Summarily, it is well recorded that the bacterial population of the post parturient uterus is not primarily responsible for metritis during the puerperium. The uterine environment predisposes to bacterial multiplication and the state of the uterine wall is instrumental in this, one feature of which is well described vis. its resistance to bacterial invasion. This will be elaborated on at a later stage.

A summary of the performance of each cow in the two groups at calving is now briefly presented, together with details of their performance during the puerperium to illustrate the complications recorded in cows in the HIGH group, and the dramatic difference between the two groups.

LOW-group

No. 1 Was in labour for hardly 30 minutes, calved without assistance and the placenta was expelled within one day.

After 7 days – Uterus still enlarged with firm tonus and clear odourless mucus. Follicular development on right ovary.

10 days – Pregnant horn slightly larger than non pregnant horn and with firm tonus. Small amount of pale brownish viscous odourless fluid escape. Large soft follicle present on right ovary.

14 days – Pregnant horn very slightly larger than non pregnant horn. Copious transparent mucus escaped on examination. Large soft CL present on right ovary.

Silent heat was diagnosed and followed by 6 completely normal cycles before she was re-inseminated and conceived.

No. 3 Calved without assistance after 2 hours labour and placenta was passed within 4 hours.

After : 3 days – Uterus enlarged without any discharge and with very firm tonus.

8 days – Pregnant horn enlarged and slightly flabby. A small amount (1/2 cup) of reddish purulent fluid escaped on palpation. Big soft follicle on right ovary.

11 days – Clear mucus reported. Right horn slightly larger than left horn. Large soft follicle on right ovary.

12 days – Ovulated on right ovary. Silent estrus recorded.

15 days – Both horns small with firm tone and equal in size. Large corpus luteum on right ovary.
The uterine wall was accidentally penetrated by the biopsy apparatus and she was eliminated from further observations.

No. 4 Right ovary embedded in adhesions following bleeding at estrus while she was a heifer.
She calved without assistance after 3 hours labour and the placenta was expelled within 24 hours.

After : 7 days – Uterus enlarged and lacked tonus but no discharge had accumulated.

11 days – Uterus much reduced in size but still lacked tonus. 1–2 tablespoons of slightly purulent mucus passed. Follicular development in left ovary.

14 days – Large soft follicle on left ovary. Both uterine horns firm and equal in size. Copious discharge, viscous and slightly milky in appearance.

18 days – Big CL in left ovary. Uterus normal in size without any sign of discharge. Silent estrus recorded on day 14.

She completed seven cycles with slightly aberrated cycle lengths (19, 28, 22, 29, 16, 22 and 21 days) but ovulatory performance normal except for delayed ovulation (day 4) at second estrus.

Follicular activity at estrus was restricted to the left ovary, which was without adhesions, in all but one estrus period at which the tissue mass

around the right ovary enlarged, propably with follicular activity, while no follicular development could be established in the left ovary.

No. 6 Calved without assistance after less than one hour in labour. Placenta was expelled after 8 hours.

After : 7 days – Uterus enlarged but tonus excellent. No discharge noticed.

11 days – Uterus much reduced in size, tonus firm and some follicular development in right ovary.

12 days – In estrus. Large soft follicle in right ovary. Pregnant horn still enlarged but firm. Copious purulent discharge about two thirds of a cup full.

13 days – Ovulated in right ovary. Discharge much more mucoid in appearance and less purulent.

16 days – Big corpus luteum in right ovary, with follicular development in left ovary. Both uterine horns equal and quite normal in size.

Her next cycle was 35 days followed by four cycles, all perfectly normal in length and ovulatory record.

No. 8 Calved without assistance after 3 hours of labour and the afterbirth was expelled within 2 hours.

After: 7 days – Uterus large with firm tonus. Copious amount of reddish brown odourless mucus was passed. Ovaries inactive.

10 days – Uterus still enlarged with reasonable tonus. Discharge copious reddish and somewhat purulent in appearance. No follicular activity.

14 days – Uterus reduced in size but pregnant horn still distinctly enlarged. Discharge copious yellowish and purulent with slightly putrid smell. Ovaries hard and inactive.

17 days – Pregnant horn firm and only slightly enlarged. Copious clear discharge with a few lumps of yellowish pus. Ovaries had become flabby and follicular development was expected.

20 days – Uterine horns equal in size and normal with firm tonus. Discharge slightly purulent but scant and mucoid.
Big follicle ruptured on slight pressure on right ovary, left ovary small and smooth. Follicular development proceeded immediately in right ovary.

30 days – Anovulatory estrus with two large follicles on right ovary, left ovary small and smooth. Mucoid discharge quite clear.

She completed 5 cycles of irregular length (23, 37, 25, 19, 20 days). Four estrus periods were anovulatory and both ovaries were enlarged with excessive follicular development. She conceived however at the 5th estrus period which was a normal one.

No. 10 Calved without assistance after one hour in labour and placenta was expelled within 2 hours.

After : 7 days – Uterus enlarged, firm with excellent tonus and no discharge.

14 days – In estrus, uterus back to normal size with abundant clear mucus.

She completed 5 perfectly normal cycles and conceived at first insemination.

No. 13 Calved without assistance after 45 minutes in labour and placenta was expelled within one day.

After : 5 days – Uterus enlarged but with excellent tonus and without discharge.

9 days – Uterus had become flabby with a bloody discharge with pieces of necrotic material with a putrid smell.

13 days – Uterus firm and much reduced in size. Discharge scant slightly bloody but mucoid and clear.

18 days – Uterus back to normal size and tonus. Ovaries small, firm and smooth.

22 days – Uterus normal size and tonus. Big soft follicle on right ovary and abundant clear discharge with a few small bits of yellowish pus.

Silent estrus was recorded. She did not ovulate however, the follicle regressed after 7 days.

After 28 days she returned to estrus and completed four normal cycles.

No. 16 Calved without assistance after $1\frac{1}{2}$ hours in labour and placenta was expelled within 6 hours.

After : 5 days – Uterus enlarged with very firm tonus and viscous honey coloured mucus.

8 days – Uterus still enlarged but flabby with some straw coloured odourless fluid.

12 days – Uterus flabby but reduced in size. Small amount of reddish purulent fluid escaped. Big follicle developed in right ovary and regressed after 4 days.

16 days – Uterus back to normal size and both horns equal. Small amount of thick yellowish purulent discharge escaped.

19 days – In estrus. Uterus firm tonus and normal size. Large follicle on right ovary and copious amount of mucu-purulent discharge.

She ovulated and completed three perfectly normal cycles.

No. 20 Calved without assistance after half an hour in labour and placenta was passed within 24 hours.

After : 7 days – Uterus well forward and large but with excellent tonus and no discharge. Follicular development in left ovary.

14 days – In estrus with a big follicle on left ovary. Uterus firm and only slightly enlarged with a copious amount of slightly bloody discharge.

16 days – Uterus back to normal size and tonus. Discharge absent. She had ovulated and a corpus luteum was present on the left ovary.

She completed 7 cycles with normal ovulatory performance but irregular length (12, 24, 12, 17, 29, 18 and 24 days) and conceived on a single insemination.

No. 21 Calved without assistance after one hour in labour and placenta was passed within 10 hours.

After : 7 days – Uterus enlarged but tonus very firm and no discharge.

14 days – Uterus much reduced in size. Tonus firm and no discharge. Follicle developing in right ovary.

15 days – Abundent milky mucoid discharge. Large follicle in left ovary. Uterus firm and pregnant horn slightly enlarged.

20 days – Big corpus luteum in left ovary. Uterine horns firm, normal in size and equal. Silent estrus recorded.

She had another silent estrus followed by a normal estrus after an estrus cycle of 38 days. This was followed by 3 perfectly normal cycles.

No. 27 Calved without assistance after 45 minutes labour and placenta was expelled within 4 hours.

After : 3 days – Uterus large and flabby without any response to handling.

7 days – Uterine tonus much improved. Clear odourless mucous with a few small bits of necrotic material. Big follicle present on right ovary.

10 days – Abundent milky mucus. Uterus much reduced in size and large soft follicle on right ovary.

14 days – Uterine horns unequal in size but firm and a few flakes of purulent mucus escaped. Big corpus luteum on right ovary.

21 days – Uterine horns firm, slightly unequal in size and no discharge present. Follicular development on right ovary.

24 days – In estrus, uterine horns perfectly normal with clear discharge.

She completed 6 normal cycles and conceived on a single insemination.

No. 29 Calved after $1^1/2$ hours labour and placenta passed within 24 hours.

After : 4 days – Uterine tonus very firm with odourless mucus and blood.

7 days – Uterine smaller, tonus very firm with reddish brown discharge.

11 days – Uterine tonus flabby and discharge reddish, purulent and watery. Follicular development on left ovary.

13 days – Uterus only slightly enlarged and tonus back to normal. Small amount of putrid watery discharge. No follicular development on either ovary.

19 days – Uterus normal size and tonus. Discharge scanty and purulent. Follicle on left ovary.

21 days – In estrus, uterus normal, big follicle on left ovary. Discharge copious and almost clear.

She did not ovulate and had a second anovulatory estrus after only 8 days followed by 6 perfectly normal cycles. She conceived on a single insemination.

HIGH-group

No. 2 Calved without assistance after 5 hours in labour and placenta was expelled within 24 hours.

After : 3 days – Uterus enlarged, good tonus on palpation and a copious amount of honey coloured fluid escaped.

7 days – Uterus far forward over brim of pelvis, flabby and lacking tonus. Odourless, reddish watery fluid with necrotic bits escaped.

11 days – Uterus reduced in size but still lacks tonus. Dark reddish and extremely putrid discharge from uterus. She walks with great difficulty and her appetite is poor. Uterine and antibiotic treatment administered.

15 days – Uterus much improved, smaller, firmer and discharge mucoid and odourless. Losing weight and gait extremely painful.

22 days – Uterus improved but still enlarged with slightly purulent mucoid discharge. Carpal and tarsal joints and both stifles swollen, hot and extremely painful and rapid deterioration in cows condition.

Although uterine involution was complete on palpation by 26 days after calving the cows condition deteriorated rapidly in spite of intensive care and treatment. She was mercifully destroyed 55 days after calving with acute fatty degeneration of the liver and severe arthritis in the carpal, tarsal and stifle joints.

No. 5 Her calf was delivered with mild traction after 8 hours labour. The placenta was expelled on the same day.

After : 7 days – The cow strains excessively. Uterus big and flabby and 1 liter of yellowish purulent fluid with a putrid odour came out on palpation. Intra uterine and a course of systemic antibiotic treatment commenced with after draining of uterus.

14 days – Cow still straining and partial vaginal prolapse has developed. Uterus much reduced in size but flabby. Discharge liquid, pungent and with yellowish necrotic particles but much improved. Antibiotic treatment continued.

17 days – Back arched and appetite poor with severe perimetritis and crepitation of thickened uterine wall. Discharge purulent but less pungent and necrotic. Material reduced in amount.

Systemic antibiotics administered over a prolonged period. General condition much improved after one week but adhesions remained around uterus and ovaries and vaginal prolapse showed regularly on straining with defecation and urination and when lying down. A chronic ulcer developed on vaginal wall. No cyclic activity for more than 90 days after calving and she was recorded as completely sterile.

No. 7 A very heavy calf (50 kg) delivered with mild traction after 6 hours in labour. Placenta passed within one hour.

After : 4 days – Uterine tonus normal and no discharge and regarded as quite satisfactory.

7 days – Small amount of odourless liquid reddish discharge. Uterus large and more flabby.

11 days – Feverish and poor appetite. Uterus far forward and lacking in tonus. Putrid fluid and necrotic pieces of cotyledonary material passed on examination. Cleaned by massaging and intra uterine and systemic antibiotics administered.

15 days – Uterus much reduced in size, thick yellow pus drained out. Follicular development on right ovary. General condition normal.

19 days – In estrus, and ovulated on right ovary. Copious milky mucoid discharge with flakes of thick yellowish pus. Uterus size slightly enlarged with firm tonus.

21 days – Corpus luteum on right ovary, uterus considered normal in size and tonus.

She cycled again at 31, 24, 22, 21 and 26 days. Uterus remained normal in size and tonus but thick purulent mucoid uterine discharge persisted for two cycles.

The second last cycle was anovulatory and at the last estrus period she ovulated at least 40 hours after insemination and conceived.

No. 9 Calved without assistance after 2 hours of labour and passed the placenta within 24 hours.

After : 7 days – Uterus far forwarded and lacking in tonus on palpation. Small amount of odourless bloody discharge escaped.

12 days – Uterus still far forwarded and lacking in tonus. Liquid yellowish purulent discharge with putrid smell massaged out.

15 days – Uterus much reduced in size with clear mucoid discharge and flakes of thick yellow pus suspended in it. Follicular development on right ovary.

22 days – Uterus normal in size with firm tonus. Big follicle was ruptured on right ovary with gentle handling. No vaginal discharge recorded.

Follicular development was recorded for 16 days before first estrus which was only 47 days after calving. Ovulation was delayed for 2 days. She cycled again at 34, 21, 21, 23 and 24 days, ovulating normally and conceived after one insemination.

No. 17 Relatively small calf was delivered with severe traction after 3 hours of labour. The calf was weak and died at birth. The placenta was expelled after a few hours.

After : 4 days – Uterus large and flabby and far forward. Large amount of reddish purulent discharge with putrid smell drained out.

9 days – Uterus far forward and flabby, straw coloured putrid fluid drained out. Appetite poor, gait very painful.

14 days – Ruminating but manure slimy with bile pigment. Losing weight rapidly and gait extremely painful. Uterus reduced but not normal in tonus and size with thick yellowish pus escaping on examination.

The cows condition declined rapidly in spite of intensive care and treatment. She died 27 days after calving with advanced fatty changes in the liver, mineralization of the myocard and severe laminitis.

No. 18 Calved without assistance after 6 hours of labour and placenta was expelled within a few hours.

After : 7 days – Uterine tonus firm with small amount of odourless viscous discharge.

12 days – Uterus reduced in size, tonus firm no discharge evident. Gait severely painful and carpal joints swollen.

20 days – Uterus firm and intra pelvic with no discharge evident. No follicular activity on either side. Laminitis and carpal arthritis severe and painful. Hock joints both swollen.

This cow lost weight and her appetite was irregular. Laminitis and arthritis of a severe degree persisted until slaughtering and by 90 days after calving no sign of onset of cyclic activity.

No. 24 The calf was posteriorly presented and delivered by mild traction after 11 hours of labour had passed. The placenta was passed within one hour after calving.

After : 4 days – Uterus large, far forward and flabby. Reddish brown putrid fluid drained out and uterine wall thickened and crepitating with severe metritis. Antibiotic and intra-uterine treatment administered and intensively maintained.

6 days – Uterine wall still crepitating but no peri-metritis. Putrid fluid with necrotic material drained out. Painful laminitis evident.

9 days – Uterus far forward, flabby and out of reach with putrid purulent fluid escaping.

16 days – Uterine size much decreased but signs of peri-metritis and slight adhesions on outer surface of uterus. Discharge thick and yellowish. General conditions improved but gait most painful.

22 days – Uterus only slightly enlarged with mucoid purulent discharge.

26 days – Uterus normal in size, discharge mucoid and clear.

She only cycled at 51 days after calving and completed 5 cycles of irregular length (17, 14, 51, 23 and 26 days), second last of which was anovulatory.

She failed to conceive at insemination and suffered severely from laminitis and arthritis in her fetlock joints.

No. 25 Her calf was delivered with mild traction after 5 hours of labour and the placenta was expelled within 6 hours.

After : 3 days – Uterus large with excellent tonus and odourless reddish discharge.

7 days – Uterus far forward and flabby with dark red discharge with putrid odour. Her gait became painful with severe laminitis. Antibiotic treatment was administered.

10 days – Uterus still flabby and far forward but discharge of improved appearance and only slightly purulent. Both fetlocks on hind legs swollen and painful.

20 days – Uterus much reduced in size and intrapelvic. Discharge still yellowish and purulent.

28 days – Uterus reduced in size, discharge still purulent. Feet severely painful and all four fetlock joints and right hock joint swollen.

34 days – Big follicle on right ovary, left ovary smooth and small. Uterus normal in size and abundant clear discharge. Silent estrus recorded.

She cycled again after 34, 23, 23 and 24 days respectively with delayed ovulations at the first two estrus periods. At slaughtering she had conceived with one live and one necrotic embrio in the uterus.

No. 30 The cow suffered a spontaneous rupture of the uterus after she had been in labour for 4 hours. A live calf was delivered from the abdominal cavity per caesarian section and a 40 cm tear was repaired after removal of the placenta.

A prolonged course of antibiotics was administered, the uterus was drained of purulent fluid at several intervals but the ovaries and uterus were embedded in adhesions following posterior peritonitis. She failed to cycle for 90 days after parturition.

No. 32 Calved without assistance after less than 2 hours labour and the placenta was expelled within 24 hours.

After : 7 days – Uterus large and far forward but with firm tonus.

14 days – Gait painful with laminitis in all four feet. Uterine size reduced and tonus flabby. Mucoid discharge clear and odourless.

21 days – Uterus only slightly enlarged with milky discharge. No ovarian activity palpated.

29 days – Uterus back to normal size.

There was no ovarian activity until she cycled at 82 days after calving. She then completed four perfectly normal cycles.

3.1.13.3 Duration of parturition and uterine involution

In Table 5 a summary is presented of the actual duration of parturition of individual cows in the HIGH and LOW groups together with the number of days taken from calving until completion of uterine involution as recorded by palpation. The number of days from calving to the development of the first corpus luteum as well as the incidence of quiescent estrus with the first estrus period after calving, are presented at the same time.

TABLE 5 – The duration of parturition, time to involution of the uterus, time to development of the first corpus luteum and the incidence of quiescent or normal estrus after calving in cows on a LOW and a HIGH level of feeding

No. of cow and group	Duration of Parturition (Hours)	Involution of uterus (days)	Development of first CL (days)	Silent or normal first estrus
LOW-group				
1	0,5	12	12	Silent
3	2,0	15	11	Silent
4	3,0	14	14	Silent
6	0,75	16	12	Normal
8	3,0	20	53	Normal
10	1,0	14	14	Normal
13	0,75	18	19	Silent
16	1,50	21	19	Silent
20	0,50	16	14	Normal
21	1,0	20	15	Silent (1st & 2nd)
27	0,75	24	10	Silent
29	1,33	14	20	Normal
Average	1,48	17,0	18,25	
SD	0,97	3,44	11,30	
HIGH-group				
2	5	26	> 55	
5	8	43	> 90	
7	6	21	19	Normal
9	2	22	47	Normal
12	–	–	–	Did not calve
14	–	–	–	Did not calve
17	3	26	> 27	
18	6,25	22	> 29	
24	11	26	51	Silent
25	5	34	59	Silent
30	4	60	> 90	
32	1,75	21	73	Silent
Average	5,20**	30,10**	60,10**	
SD	2,68	11,93	24,32	

*P < 0,05 **P < 0,01

The birth weights of the calves in relation to maternal weights immediately after calving, are presented in Table 6.

TABLE 6 – Maternal mass at calving, increase in maternal mass during treatment, calf birth mass and calf mass as percentage of maternal mass at birth in cows on a LOW and a HIGH level of feeding

Cow No. and group	Maternal mass (kg)	Increase in maternal mass (%)	Calf birth mass (kg)	Calf as % of cow mass
LOW-group				
1	463	11,6	22,7	4,9
3	468	29,6	32,6	7,0
4	503	21,5	29,5	5,9
6	488	11,2	32,6	6,7
8	506	4,1	32,6	6,5
10	527	8,2	32,7	6,2
13	477	12,8	37,2	7,8
16	525	5,8	33,1	6,3
20	530	16,5	33,1	6,2
21	452	10,8	24,5	5,4
27	483	17,2	32,2	6,9
29	530	12,8	36,3	6,9
Average	496	13,6	31,7	6,4
SD	26,9	6,7	3,9	0,7
HIGH-group				
2	585	38,6	31,8	5,4
5	651	49,3	32,6	5,0
7	607	50,2	44,9	7,4
9	655	43,0	32,7	5,0
17	674	57,5	34,5	5,1
18	592	36,4	29,5	5,0
24	649	38,9	36,7	5,7
25	630	28,3	41,7	6,6
30	702	39,2	42,2	6,0
32	636	28,2	42,2	6,6
Average	638**	40,9**	37,1**	5,8*
SD	34,5	8,9	4,9	0,8

*P < 0,05 **P < 0,01

At calving all dystocia cases could be classified according to the amount of traction required for delivery of the calf *vis. mild traction* where delivery was effected with the aid of a single assistant and little effort or *severe traction* where delivery was possible only on traction by more than one assistant and considerable effort.

Ease of calving is accordingly presented in Table 7 together with the birth mass of the calves expressed as a percentage of maternal mass as recorded immediately after birth.

TABLE 7 – Ease of calving of Friesland cows on HIGH and LOW feeding levels in relation to the mass of calves at birth expressed as a percentage of the maternal body mass immediately after calving

	Number of cow	Mass of calf (kg)	Calf mass as % of cows mass	Average %
HIGH-group				
Mild traction	5	32,7	5,0	
	7	44,9	7,4	
	24	36,7	5,7	
	25	41,7	6,6	6,17
Severe traction or caesarian	17	34,5	5,1	
	30	42,2	6,0	5,6
Unassisted at birth	18	29,5	5,0	
	2	31,8	5,4	
	9	32,7	5,0	
	32	42,2	6,6	5,5
LOW-group				
All unassisted at birth	1	22,7	4,9	
	3	32,7	7,0	
	4	29,5	5,9	
	6	32,7	6,7	
	8	32,7	6,5	
	10	32,6	6,2	6,4
	13	37,2	7,8	
	16	33,1	6,3	
	20	33,2	6,2	
	21	24,5	5,4	
	27	32,2	6,9	
	29	36,3	6,9	

Incorrect fetal presentation was never recorded to cause dystocia. Only one calf in the HIGH group was posteriorly presented and delivered by mild traction.

It is clear from Table 5 that the *ad libitum* level of feeding prolonged parturition time significantly ($P < 0,01$) from an average of 1,48 $\pm$ 0,97 hours in the LOW group to 5,20 $\pm$ 2,68 hours in the HIGH group. Body mass at the time of calving as well as the percentage increase in body mass during the duration of

the feeding treatments, were significantly higher in the *ad libitum* feeding group ($P < 0,01$) as shown in Table 6. The correlation between body mass and duration of parturition was, although insignificant, higher in the LOW group ($r = 0,314$) than in the HIGH group ($r = -0,718$). Individual variation within groups and particularly in the HIGH group, overshadowed the effect of body mass. In this respect too, the incidence of uterine inertia to be reported on the HIGH group, obviously played an important role.

The increased incidence of dystocia in the HIGH group according to Table 7 must be seen as one of the most severe disadvantages of *ad libitum* or high level feeding in the bovine. This was followed by a highly significant increase ($P < 0,01$) in the time taken to completion of uterine involution of $17,0 \pm 3,44$ days in the LOW group as against $30,10 \pm 11,9$ days in the HIGH group.

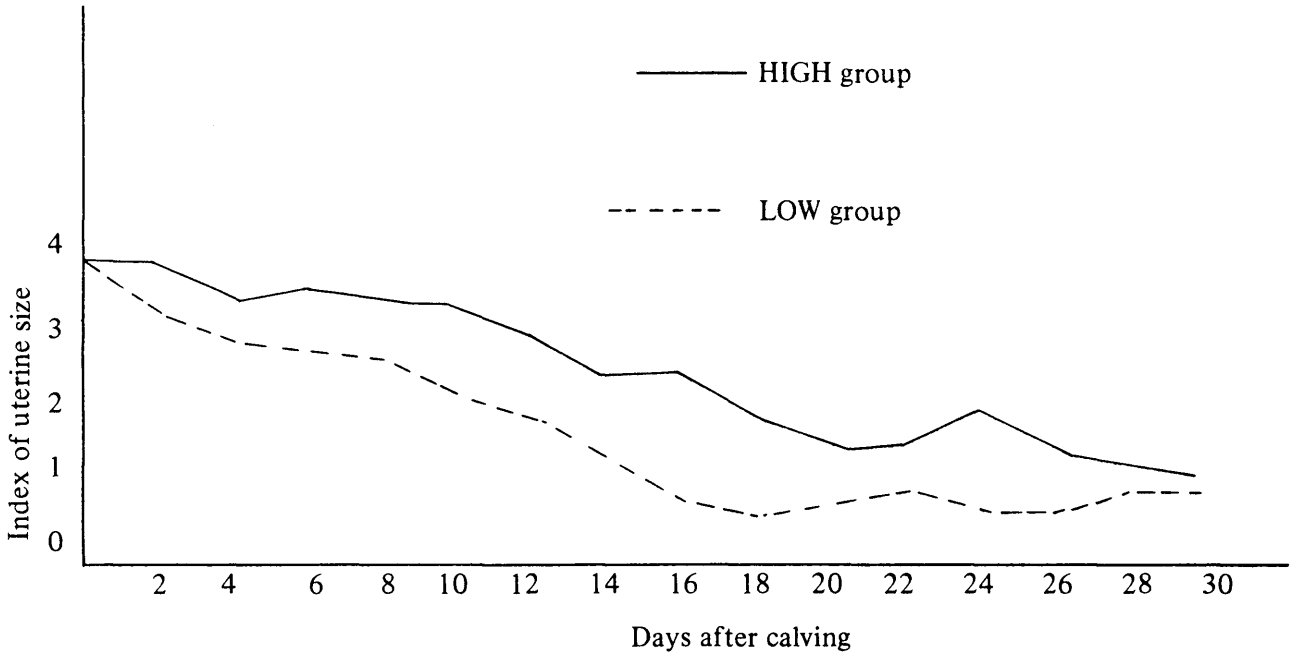
Delayed uterine involution by *ad libitum* feeding is further illustrated in Figure 8 where the size of the post parturient uterus was determined by rectal palpation at 3 to 4 day intervals and expressed to the following numerical index:

4 – Size of the uterus immediately after calving
3 – Size of the uterus at 4–5 months pregnancy
2 – Size of the uterus at 3 months pregnancy
1 – Size of the uterus at $2–2^1/2$ months pregnancy
0 – Size of the normal non-pregnant uterus

It is clear from this illustration that the rate of uterine involution in the HIGH group was not only delayed, but that the uterine size remained enlarged throughout. The correlation between maternal body mass at calving and the period to completion of uterine involution in the HIGH group was accordingly positive at $r = 0,634$ which approached significance ($2 P < 0,975$) as against an insignificant correlation in the LOW group ($r = 0,035$).

The creation of favourable conditions for the development of metritis by delayed uterine regression will be elaborated on later. In this study 8 out of the 10 cows that calved in the HIGH group accordingly required treatment in the form of draining of lochia from the uterus and local or systemic antibiotics in spite of which it will be shown that the fertility of cows in this group was severely reduced.

FIGURE 7 – Decrease in the size of the uterus after parturition as determined by rectal palpation and expressed according to a numerical index for the HIGH and LOW groups respectively



The effect of the duration of parturition on uterine involution was considered. Although differences between groups were highly significant ($P > 0,01$) as indicated in Table 5, correlations were insignificant in the LOW ($r = 0,188$) as well as in the HIGH group ($r = 0,084$). This once again emphasizes the importance of individual variations encountered throughout this investigation. It is felt however, that studies on larger numbers of animals might produce different results.

Likewise, although the birth mass of calves in the HIGH group was significantly higher ($P < 0,01$) than in the LOW group, the correlation between fetal mass and uterine involution was insignificant in the LOW ($r = 0,194$) as well as in the HIGH group ($r = 0,084$).

From the individual histories of the cows in the HIGH group it can further be seen that it was quite a regular feature for the placentae to be expelled within the normal time after calving and for uterine recovery to take an apparently normal course for the first 2 to 4 days. This was apparent from the firm tonus of the uterus after calving and from the rapid contraction of the longitudinal layer of the endometrium on palpation as well as the clear and odourless lochia at this stage.

This was however often followed by deterioration and metritis after a week or two, characterized by a total loss of uterine tonus with accumulation of lochia of greater fluidity and putrefaction with septicaemia.

Post parturient metritis was indeed of such severity, that perimetritis or posterior peritonitis was a common sequel as shown and it was frequently followed by permanent sterility.

Deterioration in the incidence and severity of laminitis and arthritis was undoubtedly brought on by the post parturient septicaemia. In addition to this, liver digeneration, peritonitis, pleurisy and endocarditis were reported and will be discussed at a later stage in relation to puerperial infection.

During the puerperium, infection of the uterus is, not incorrectly, viewed as analogous to wound infection with organisms entering via the cervix.

According to Jubb & Kennedy (1971) 25 to 30 per cent of cows are infected in the normal puerperium but most of them recover spontaneously from infection with Corynebacterium pyogenes, Escherichia coli and other bacterial mixtures. Accumulation of lochia however, provide an excellent environment for all types of bacterial growth and its retention beyond the normal period is particularly favourable for the most devastating amongst them, the anaerobes.

The two main ingredients of involution are contraction to obliterate the uterine cavity and reconstitution of the epithelium.

Retarded post partum contraction is an index of diminished myometrial tonus, which according to Jubb and Kennedy (1971) may be an exhaustion phenomenon consequent on prolonged dystokia.

The greatest volume of lochia, a mixture of blood, fetal fluid, mucus and maternal or placental detritus, is present in the first 48 hours after parturition and is rapidly removed by discharge and resorption. All factors favouring the accumulation of an excessive volume of lochia and its retention beyond the normal period, provide an excellent environment for bacterial growth.

Reports vary considerably on the time taken from parturition until completion of uterine involution, particularly as the end point for determining uterine involution is not sharply defined.

Roberts (1956) reported normal uterine size on palpation after 18 to 20 days with only a few ml of lochia still present after 18 days. Histological changes however, proceeded until 40 to 50 days after calving.

Retarded involution is described to follow uterine inertia, dystocia or retention of the fetal membranes and this is usually followed by puerperal metritis within a few days after birth (Arthur, 1964).

Marion, Norwood & Gier (1968) obtained data by rectal palpation on 385 cows and found that involution was complete in pluriparous cows at 40,6 days and in multiparous cows at 34,0 days after calving, the difference being highly significant.

Casida and his co-workers (1968) found uterine involution to be complete by 30 days post partium with a difference between suckled and non suckled cows

on histological examination while Wagner & Hansel (1969) again reported that the presence of suckling calves or anaemia did not delay involution. According to Oxenreider & Wagner (1971) neither lactation nor energy intake appear to affect normal uterus involution although post partum follicular activity was delayed by low energy intake and lactation.

Buch, Tyler & Casida (1955) and Tennant & Peddicord (1968) demonstrated that uterine involution in Holstein Friesian cows was not complete until 42 to 50 days as judged by weekly palpations to determine uterine tone and consistency, size and location of the uterus. Abnormal calvings, including twins, retained placentae, dystocia, prolapse, abortions and dead calves resulted in involution taking 5 days longer.

An analysis of fertility of cows bred at different stages post partum led Buch, Tyler & Casida (1955) to conclude that involution of the uterus was necessary for conception after parturition while Tennant & Peddicord (*op. cit.*) failed to demonstrate any relationship between fertility and the involutionary status of normal uteri. Metritis however, caused a delay in involution with a significant reduction in fertility.

The breeding efficiency of beef cows in relation to the involutionary state of the uterus as determined by rectal palpation and in relation to post partum interval, was studied by Perkins & Kidder (1963). They reported no significant difference in conception rate between involuted and non-involuted cows but that the length of the post partum interval might be of greater significance.

It should be remembered however, that the state of the musculature of the post partum uterus is all that can be determined by palpation, whereas the tissues below the musculature must reasonably be expected to exert a far greater influence on fertility.

According to the results recorded by Gier & Marion (1968) uterine epithehal covering was complete at 25 days after parturition but muscular contractions and shrinkage of blood vessels proceeded until 40 to 50 days post partum.

Summarily therefore, uterine size was back to normal on palpation from 18 to 30 days after calving, with suckling, dystocia, retention of the placenta and

metritis having an influence on the length of this period. Histological changes proceeded after clinical changes were complete.

In the present study the effect of prolonged parturition as brought on by the higher level of feeding was clearly demonstrated.

3.1.13.4 Uterine inertia

Uterine inertia or the absence or feebleness of uterine contractions at or subsequent to parturition is a well known phenomenon in animals giving birth to large litters like dogs or pigs.

In bovines however, it is not well recorded particularly because of the intricacies and the amount of time involved in the study of the birth process in individual animals.

In this study evidence can be presented on the possible incidence of uterine inertia at parturition in the HIGH group.

First of all, the possible causes of the higher incidence of dystocia in the HIGH group have to be considered. It is clear from Table 6 that although the absolute size of the calves in the HIGH group was more than in the LOW group, the relative size of the calves in the LOW group was more than in the HIGH group.

Evidence is available however, that bodyweight of the newborn in relation to maternal weight at birth in bovines, is a crucial factor in dystocia (Reyneke & Penzhorn 1964, Reid *et al.* 1964, Amir, Kali & Volcani 1967, Monteiro 1969).

According to Wiltbank (1972) dystocia in heifers on a high feeding level was not because birth weight was increased. Birth weight was not increased by high feeding levels but it was decreased if heifers do not receive adequate feeding. He further states that the amount of fat in the pelvic region could have decreased the size of the pelvic opening.

Gestation length in this study varied between 264 and 282 days which fits in with the normal gestation length of dairy cattle (Salisbury & Vandemark, 1961) and could hardly have been a factor in the incidence of dystocia.

In this investigation the actual birth mass of calves in the HIGH group was significantly higher ($P < 0,01$) at 37,1 ± 4,9 kg than that of calves in the LOW group at 31,7 ± 3,9 kg. As a percentage of maternal mass at calving however, the average birth mass of calves in the LOW group was higher ($P < 0,05$) at 6,4 ± 0,7kg as against 5,8 ± 0,8kg of calves in the HIGH group. The high incidence of dystocia in the latter group therefore indicates that in this investigation, calf mass in relation to maternal mass at birth did not account for difficulties at birth. The correlation between maternal body mass and fetal birth mass was consequently insignificant ($r = 0,571$ in the LOW group and $r = 0,242$ in the HIGH group). Likewise, the correlation between fetal birth mass and duration of parturition was insignificant in the LOW ($r = 0,255$) as well as in the HIGH group ($r = -0,136$).

This clearly infers that dystocia in this study was related to factors other than maternal or fetal size *per se*. Out of all the dystocia cases in the HIGH group in this respect, only two cows, numbers 17 and 30 (Table 7) required severe traction. In cow 17, the actual as well as the relative mass at birth of the calf was well below the average of the group. In cow 30, the actual and the relative mass at birth of the calf was conducive to dystocia and spontaneous rupture of the uterus resulted after only 4 hours of labour. This is an extremely rare condition in the bovine and reminiscent of an inherent weakness in the uterine wall. In no less than 4 cows in the HIGH group, dystocia was recorded after prolonged periods of labour and yet, only mild traction was required for delivery. These cases particularly are reminiscent of the absence or feebleness of uterine contractions and were recorded as primary uterine inertia.

It has been indicated that the pelvic opening might be reduced in size by adipose tissue in obese animals. The role of intrapelvic fat in dystocia can hardly be measured with any accuracy in the live animal. On the strength of the individual histories mentioned above, it was felt however, that mechanical interference by fatty tissue in the pelvic outlet, could not be ascertained.

Cognisance was also taken of the role of pelvic conformation in dystocia. In the present study this was disregarded on the grounds of the complete absence of dystocia in the LOW group and its frequent incidence in the HIGH group, and in addition, again, with certain individual histories mentioned above in mind.

Arthur (1964) mentions lack of excercise and excessive fatness as possible causes for uterine inertia in cattle. According to Roberts (1957) primary inertia is rare in the bovine and is seen more often in old cows and in animals that are closely confined and hence lack excercise. He states that it is more common in dairy than in beef cattle and is associated with over fatness as well as debility and hormonal imbalances.

It is suggested that with more precise techniques to diagnose primary uterine inertia in cattle, a higher incidence of this condition will be recorded than presently particularly with intensification and high feeding levels in dairy animals.

It is quite obvious according to Table 5, that in any commercial herd, the difference between time taken from calving until the first estrus or the first corpus luteum, would have been ruinous to economic production. The LOW group developed the first corpus luteum at 18,25 ± 11,30 days after calving. In the HIGH group, this period was 60,10 ± 24,32 days, a highly significant ($P < 0,01$) difference and an extremely long average for the dairy cow. This illustrates the profound effect of stressors during the puerperium on the fertility of cattle.

Reports are available on the effects of various post calving stressors on bovine fertility. Gier & Marion (1968) reported that in dairy cattle the number of days from parturition to first ovulation, is significant between high producers and low producers.

A report from the Willow Francis Group (1971) shows that abnormal cows with diseases near calving such as milk fever, ketosis and retained placental membranes, produced more milk than did normal cows with no disease problems at calving. Normal cows however, had their first estrus 15 days after calving whereas abnormal cows did not come into estrus until 34 days after calving. The interval from calving to first estrus increased directly with the level of production in abnormal cows, but in normal cows production had no effect on onset of first estrus.

Morrow, Roberts & McEntee (1969) studied post partum ovarian activity in normal cows and in abnormal cows that had abortion, dystocia, retained fetal membranes, metritis, milk fever, acute mastitis, ketosis or other debilitating diseases. They reported a post partum period until first estrus of 15 days in normal and

34,4 days in abnormal cows. They ascribe part of the difference to possible nutritional effects because of anorexia due to chronic ketosis, a condition never diagnosed in this study.

Several studies confirm that the mere physical presence of debris in the involuting uterus inhibit both neural and hormonal feedback mechanisms regulating the control of normal cycles, and that estrus cycles are shorter than normal when the uterus is dilated during the early phase. (Callaghan *et al.* 1971 quoting Yamauchi & Nakarah; Hansel & Wagner 1960.)

3.2 Histological examination of the endometrium

It is clear from the foregoing report that profound differences exist in the rate and nature of uterine involution between cows in the HIGH and in the LOW groups. Whilst it is not possible to interpret these directly in terms of the different nutritional levels instituted for this study, there is little doubt that the prolongation of parturition time and the incidence of dystocia played important roles.

It is common procedure too, that many dairy herds are submitted to routine metritis treatment after calving and that *post partum* metritis is an important cause of prolonged calving intervals and permanent sterility due to perimetritis and oophoritis. The control of this problem is generally directed towards the improvement of therapeutic procedures while preventive measures in the bovine are directed against the known specific venereal diseases.

No reports have been submitted in this country on the aetiology or predisposing causes of post parturient metritis in cows, which is empirically regarded as a bacterial infection *per se.*

It has however been observed over many years and in a large number of cattle that non-specific post parturient metritis is restricted to a considerable extent to well fed dairy herds under intensive systems. Although controlled data are not available, the impression was gained that the incidence of non-specific metritis is much lower in dairy herds on extensive systems and even in poorly fed herds, while the condition is, comparatively speaking, non-existent in beef herds.

Dawson (1950) provided a summary of the various factors that might contribute to puerperal metritis and he was the first to associate the high incidence of metritis to "occupational stress" in dairy cows.

He mentions that dietary imbalances might lead to pathogenic activity in normally saprophytic organisms in the bovine uterus and that lack of exercise, excess concentrate and peak milk yields might be consistent with a high incidence of metritis. He also reports a "causeless" incidence of retained placentae and purulent endometritis in high yielding cows.

Dawson further quotes Hignett's results who reported a higher incidence of metritis in hypothyroid than in normal bovines. This was confirmed by Filsinger (as quoted by Dawson) who distinguished 250 hyperthyroid and 400 hypothyroid types among 1 600 cows with a significantly higher incidence of endometritis among hypothyroid types.

Evidence will be presented on the remarkable ability of the normal bovine uterus to overcome bacterial invasion.

It will be shown however that prolonged labour which was evidently associated with parturition damage to the uterine wall, delayed the return to normal of the population of the various cell types in the endometrium.

As a basis to the understanding of the cellular changes in the uterine wall during involution, a review of the cellular composition of the normal bovine uterine wall is now presented.

3.2.1 The morphology of the non-pregnant uterine wall

The bicornual uterus of the cow joins posteriorly into a short corpus uterus of 2 to 4 cm in length and into this enters the spiral shaped canalis cervicis, the external orifice of which projects into the vaginal cavity for a distance of about 3 cm.

The uterine wall consists of several confluent layers:

(i) The perimetrium or serosa is a thin outer covering of semi-dense collagenous tissue which is covered by peritoneal mesothelium except in the posterior portion.

(ii) The myometrium, or tunica muscularis, is divided into a thick inner circular and a thinner outer longitudinal layer. The stratum vasculare, which contains numerous large blood vessels and nerves, lies between the two muscular layers.

(iii) The inner layer, or endometrium, is divided into cotyledons, which are the sites of attachment of the fetal membranes to the uterus, and the inter-cotyledonary or intercaruncular areas.

According to Skjerven (1956), the term cotyledon refers to either maternal or fetal placenta or the union between them which is also referred to as a placentome, as against the term "carnucle" which refers to only the maternal part of the placenta.

Where reference is made to only the maternal portion of the endometrium, the term "intercaruncular area" is therefore the correct term for the non pregnant uterus.

During pregnancy, villi of the fetal chorion project into crypts in the epithelial surface of the carnucular area to form the placentome. The epithelium of the placentome remains intact during pregnancy while the intercotyledonary epithelium degenerates (Trautman & Febieger, 1957).

The endometrium consists of several subdivisions.

3.2.1.1 *The epithelium* is tall, columnar and occasionally ciliated (Trautman and Febieger 1957).

Dawson (1950) sums up the reports of various investigators and concludes that although some observers claim pseudostratification of the epithelial layer, various others point out that an illusion of pseudostratification can be produced by section at an acute instead of a right angle to the plane of the surface. They claim that the epithelium consists of a single layer of cells, with cell height, vesicularity of the cell and nuclear volume, depending on the stage of the cycle.

Marinov & Lovell (1968) describe the surface epithelium as pseudostratified columnar during most stages of the cycle and low columnar during estrus.

The distinction between the caruncular and intercaruncular areas is not clear although differences in the time and magnitude of changes during the cycle have been reported (Weeth & Herman 1952, Marinov & Lovell 1968).

3.2.1.2 The lamina propria consists of a stratum compactum and a stratum spongiosum.

3.2.1.3 The stratum compactum is a comparatively narrow zone of densely cellular connective tissue with the precursors of plasma cells and histiocytes, which it forms in tissue culture, according to Dawson's summary above. The stratum compactum is situated immediately below the basement membrane of the surface epithelium, and it is characterized by a cyclic variation in the population of polymorphonuclear leucocytes and lymphocytes. Histiocytes, which often contain pigment from degenerating blood elements, plasma cells, and large numbers of mast cells, are regular features of the stratum compactum.

3.2.1.4 The caruncles are thickenings of the stratum compactum and are more densely cellular and contain more blood vessels than the intercaruncular area.

3.2.1.5 The stratum spongiosum contains more loosely arranged connective tissue and cells forming trabeculae which extend into the myometrium.

3.2.1.6 The uterine glands are contained in the stratum spongiosum. They are branched coiled tubular glands terminating at the myometrium or deep in the stratum spongiosum.

As the glands pass superficially, they become straighter and their lumina become larger. The gland necks do not penetrate the thickened stratum compactum of the caruncular area but they take a diagonal course around this area.

Coiling of the glands, glandular epithelial size and lumina of the uterine glands vary according to the stage of the cycle, while glandular activity, as reflected by epithelial cell height and nuclear volume and density, is bigger towards the surface epithelium than towards the myometrium Weeth and Herman 1952, Dawson 1950).

Marinov & Lovell (1968) reported great variation in histochemical activity between glands close to the surface and those deep in the stroma. Skjerven (1956)

suggested that these changes in the glands seemed to be decided by the individual rhythm of the cells rather than the stage of the cycle.

The glandular lumina might be empty or contain varying amounts of detritus, often cellular in appearance, over the cause of which there appears to be some uncertainty.

Weeth & Herman (1952) express the opinion that the walls of hypertrophic glands might collapse so that when these are examined microscopically, islands of glandular epithelium are often seen in the lumina. The cellular debris according to Johnson (1965) is unattached to the epithelium with variations in appearance at different stages of the cycle, an opinion supported by Marinov & Lovell (1968)

De Bois (1961) quotes Hilty and also Rasbeck who both reported that the glandular epithelium degenerates and that the degenerated epithelium is desquamated into the lumina together with lymphocytes while through mitotic proliferation a new epithelial covering to the now much smaller glandular diameter is provided.

3.2.1.7 Wandering cells

In this investigation particular attention was paid to the presence of wandering cells in the endometrium biopsy specimens. A thorough understanding of their presence and their significance in the normal bovine endometrium, is essential for the purposes of this study and is now presented on neutrophiles, lymphocytes, eosinophiles, plasma cells, histiocytes and mast cells.

3.2.1.7.1 Neutrophiles, according to several workers, (Skjerven, 1956; Dawson, 1950; de Bois, 1961) are present in the follicular phase of the cycle, often in large, numbers but varying, between a couple of days before and after estrus.

Hirsch (1965) summarizes the role of neutrophiles in host resistance to infectious disease by phagocytosis of bacteria and inanimate objects. He also discusses the role of neutrophiles in the production or severity of the inflammatory process itself by the action of lysosomal enzymes from the granules of polymorphonuclear leucocytes, the so-called Schwartzman-like reactions, which can be prevented by pre-treatment of animals with cortisone.

Several reports are on record on the resistance of the uterus to bacterial infection during the estrus phase (Chang, 1967) while van Waveren (1962) studied

spontaneous recovery in puerperal metritis in bovines and reported a most favourable reaction in comparison to controls on antibiotics. Both reactions illustrate the role of neutrophiles in the endometrium.

3.2.1.7.2 *Lymphocytes* in comparison to the other cell types occur more regularly throughout all stages of the cycle in reproductively normal cattle according to de Bois (1961). Skjerven (1956) summed up various records and reported an increase in the number of lymphocytes during di-estrus and 2 to 3 weeks after calving with migration through the uterine epithelium during this stage. From his own observations he recorded small numbers of lymphocytes in the endometrium of reproductively normal cattle, both in the stroma and in the surface epithelium.

He also recorded well demarcated round to oval foci of lymphoid tissue in some of the animals studied by him. These foci occurred to the same extent in cows that were infected during the puerperium, than in cows that were not infected.

The participation of lymphocytes in immune reactions is well recorded although the mechanism of these reactions remains to be classified according to Gesner (1965). He also relates that the role of lymphocytes as a source of macrophages or fibroblasts or multi-potential stem cells has not been proved, but he concedes that lymphocytes might contribute to bodily functions in ways unrelated to immunological activities.

3.2.1.7.3 On *eosinophiles* reports vary considerably about their occurrence in the endometrium. De Bois (1961) recorded irregular numbers of eosinophiles in the endometrium and concluded that although their function is uncertain, their presence do not indicate an abnormal condition.

Dawson (1950) reported an age difference in his observations and quoted Weber who recorded eosinophiles as rare in the endometrium. Skjerven (1956) on the other hand, observed a "remarkable infiltration" of eosinophiles in the endometrium with a "peculiar" distribution during the cycle, but he recorded no obvious tendency for biological variation in the number of eosinophiles, a finding supported by other workers according to Skjerven. He concludes that eosinophiles possibly reflect a more general variation in the adrenal cortical activity in individual animals.

The present state of our knowledge on eosinophiles is summed up by Hirsch (1965). The wide variety of hypotheses on eosinophile function reflect fact that no role of these cells in mammalian physiology has as yet been established firmly. Antigen/antibody reactions attract eosinophiles and allergies, parasitic infestations and skin diseases are responsible for changes in blood and tissue levels of eosinophiles but no known concept of eosinophile function explains these changes. The well recorded association between eosinophiles and adrenal function has been of some value in the assessment of adrenal function, but does not shed any light on the function of eosinophiles.

From the relationship between eosinophiles and histamine, there is some support for an antihistaminic function of eosinophiles but sufficient evidence is not available to confirm an anti-inflammatory role for eosinophiles in vivo.

3.2.1.7.4 *Plasma cells* were recorded in some biopsy specimens of the endometrium studied by Skjerven (1956) and de Bois (1961). When present they were often grouped and distribution was throughout the stroma of the endometrium but not in the epithelium. They found a large number of plasma cells in a few individual biopsies, especially those from older cows where the highest concentration of plasma cells was superficially in the endometrium. Skjerven (1956) recorded a highly significant increase in the number of plasma cells with increasing age in cows.

Dawson (1950) summing up the results of several workers, reported that plasma cells are rarely recorded in heifers with a steady increase with age, where their presence with lymphocytes is interpreted as a local immune reaction to repeated post puerperal infections.

Uhr (1965) gives an account of the role of plasma cells in immunology.

Plasma cells in the spleen and lymph nodes are producers of antibodies together with small lymphocytes. The relationship between these two cell types is unclear. Antibody formation outside lymphoid tissue in organs such as the uterus, mammary glands and bladder as well as an immunologic memory, has been demonstrated in plasma cells. In germ free animals, no plasma cells could be demonstrated in the intestines and spleen.

3.2.1.7.5 *Histiocytes* or macrophages can be identified by their content of pigment and cell rests and they occur, according to the results obtained by several workers (Dawson, 1950; de Bois, 1961) in small numbers in the endometrium and at all stages of the cycle.

Skjerven (1956) recorded large numbers of histiocytes in the stratum compactum 100 to 200 μ beneath the epithelium and in the neighbourhood of capillaries, where they often occurred in groups. He also recorded no cyclic variation or age incidence in the number of histiocytes in the endometrium.

3.2.1.7.6 *Mast cells* are reported in the stroma of the stratum compactum in all endometrium biopsies 75 to 100 μ beneath the surface epithelium (Skjerven, 1956; Dawson, 1950; de Bois, 1961).

The presence of mast cells in the rest of the endometrium vary greatly with localized accumulation of the cells and with a concentration near blood vessels according to Skjerven (1956).

There is general agreement that variations in the numbers of mast cells occur, irrespective of the stage of the cycle.

Mast cells are responsible for histamine, serotonin and heparin metabolism.

Bloom (1965), in a summary of the characteristics of mast cells, concluded that plasma and tissue heparin function homeostatically to regulate protein interactions, and that heparin, as an ion exchange agent, inactivates noxious substances carrying cationic groups by complex formation with polysaccharides.

Bloom also provides evidence that mast cell heparin takes part in clearing hyperlipaemia and that mast cell histamine in some species might be analogous to serotonin in others, and that it constitutes the major inactivator of increased capillary permeability, smooth muscle contraction and phagocytic stimulation, while mast cell proteolytic enzymes perpetuate vascular responses in the elimination of noxious matter.

3.2.2 Involutional changes in the bovine uterus after parturition

Uterine involution was studied in clinically normal post partum dairy cows by Gier & Marion (1968).

A rapid decrease in size of the post gravid horn due to vasoconstriction and muscular contraction was recorded during the first few days after parturition. The length of the post gravid horn was reduced to half the parturition size by 15 days and to a third by 30 days after calving. By 50 days the normal involutionary process was complete and uterine mass had decreased from 9,0 kg at birth to 1,0 kg at 30 days and 0,75 kg at 50 days post partum.

Necrosis of the caruncula by 5 days after calving resulted in septal disorganisation and leucocyte infiltration. By 12 days most caruncular septa had sloughed, leaving a raw surface with protruding remnants of blood vessels. By 15 days, sloughing was complete to the stratum compactum and the caruncular surface was covered by epithelium at 25 days.

Muscular shrinkage continued to reduce the size of the uterus and pregravid size was reached by 40 to 50 days after parturition.

Catchpole (1969) asserts that clinical and veterinary literature describe uterine involution almost exclusively in pathological terms in which processes like cell lysis, necrosis, hyaline change and fatty degeneration figure prominently and with scant reference to birth trauma in the larger domestic animals.

Various factors however play a role in the involutionary process, one of which has already been mentioned earlier on in this report on the effect of length of parturition on involution. This will be discussed further in the light of histological findings to be reported.

Casida (1968) after a series of studies, reported that suckling brought about a decrease in the endometrial glandular mass and in the lymphocyte count, while the interval since calving increased the polymorphonuclear leucocyte counts and lessened the severity of experimentally induced endometritis. He also reported that the large physical size of the uterus after calving, the sloughing of the cotyledons and the removal of debris and the high counts of infiltrating cells – lymphocytes, polymorphonuclear leucocytes and histiocytes – were all related to lower fertility.

The difference between the two uterine cornu was reported on by Riesen and his associates (1968). There was no difference in the intercaruncular epithelium during the first 30 days post partum, but the caruncular epithelium was re-established at a later stage in the pregnant than in the non pregnant horn.

3.2.3 Cyclic changes in the endometrium

The non gravid uterus is changed relatively little during the estrous cycle when gestational changes are compared with those seen in the non-gravida according to Weeth and Herman (1952).

Several reports (Skjerven, 1956; Wordinger, Dickey & Hill, 1970) relate an increased phospatase activity to the progesterone level of the organism and an increased glycogen activity to the estrogen level. These variations are evident during the follicular and luteal phases of the cycle.

No variations are obvious in the endometrial glands between the follicular and luteal phases, neither did Skjerven (1956) succeed in demonstrating any cyclic variation in histochemical properties of the glands. He reported an individual rhythm of the glandular activity and an increased activity of glands close to the surface compared to glands situated deeper in the endometrial stroma.

3.2.4 Uterine biopsies as a method of examination of the endometrium

Loss of epithelium and tattering of tissue presented a major problem with the collection and subsequent treatment of biopsy specimens. Meticulous sharpening and tempering of the cutting edge turned out to be essential in the cutting of neat specimens.

The function of the biopsy instrument was improved by providing a surface of soft metal alloy to cut on and this helped to preserve the sharpness of the cutting edge.

No problem was encountered in passing the biopsy instrument through the cervical orifice, even during the luteal stage. The uterine wall was penetrated only once. A localised perimetritis developed which cleared up readily with systemic antibiotics but adhesions remained and the cow had to be eliminated from further biopsies.

No cow suffered any obvious symptom of discomfort when biopsies were taken and no symptom of any bacterial infection was ever observed as a result of the operation.

In all except a few cases it was succeeded in to embed specimens at the correct angle to cut sections through the epithelium and underlying tissues. Where this was not succeeded in, a further series of sections had to be cut at a different angle.

In all biopsy specimens the epithelium and underlying compacta and stratum spongiosum were included together with the endometrial glands and often small portions of the tunica muscularis. With only a few exceptions, specimens were obtained from the intercaruncular areas of the endometrium presumably because of the small and rapidly degenerating caruncular area.

The studies of Skjerven (1956), Kapelmacher (1954) and de Bois (1961) confirmed the suitability of uterine biopsies to study the histology of the bovine endometrium. De Bois presented evidence that with the possible exception of 5 per cent of specimens a single biopsy specimen was representative of the endometrium of the entire uterus. He also showed that the resistance of the uterus to bacterial infection during the follicular phase make it possible to take biopsy specimens at the time of insemination. The biopsy operation up to 13 days after a fertile service does not interfere with conception, neither does the presence of blood in the uterus after a biopsy interfere with semen fertility.

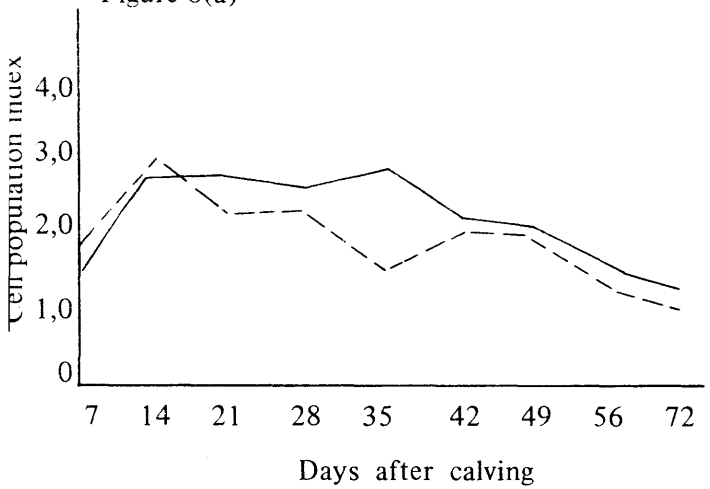
3.2.4.1 The cell population of the endometrium in the HIGH and LOW feeding groups

The stratum compactum of the endometrium contained a larger number of mobile cells of all the different cell types studied during the puerperium and the various stages of uterine involution, as well as during the subsequent estrus cycles, inclusive of both the follicular and the luteal phases of the cycle.

Figures 8(a) – 8(f), illustrate the respective cell populations, according to the numerical index employed in this study for cell types, at weekly intervals from the 7th to the 72nd day after calving for the HIGH and the LOW groups respectively.

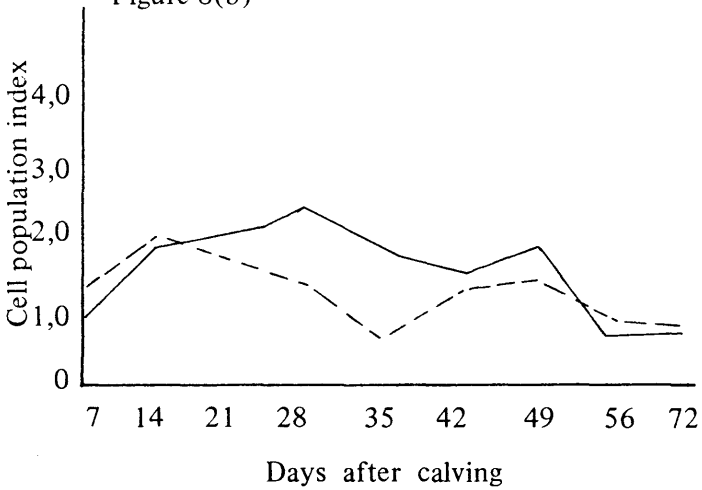
The following observations are clear from Figures 8(a) – 8(f) in relation to the various cell types.

Figure 8(a)



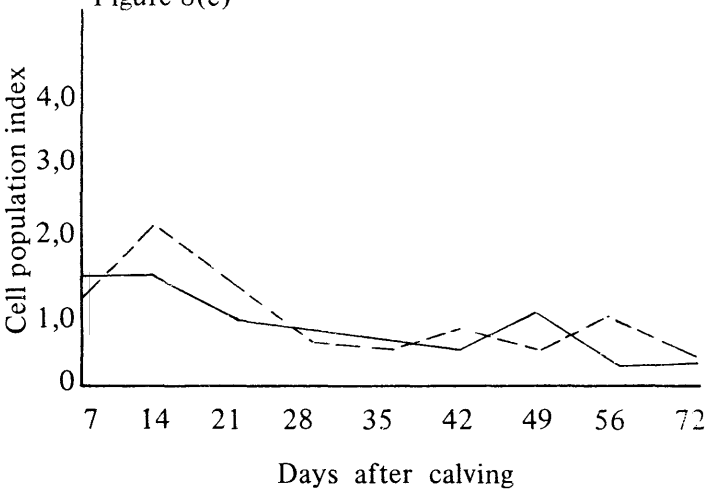
LYMPHOCYTES

Figure 8(b)



PLASMA CELLS

Figure 8(c)



MAST CELLS

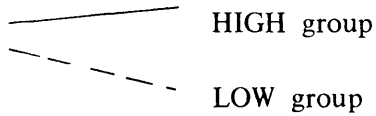
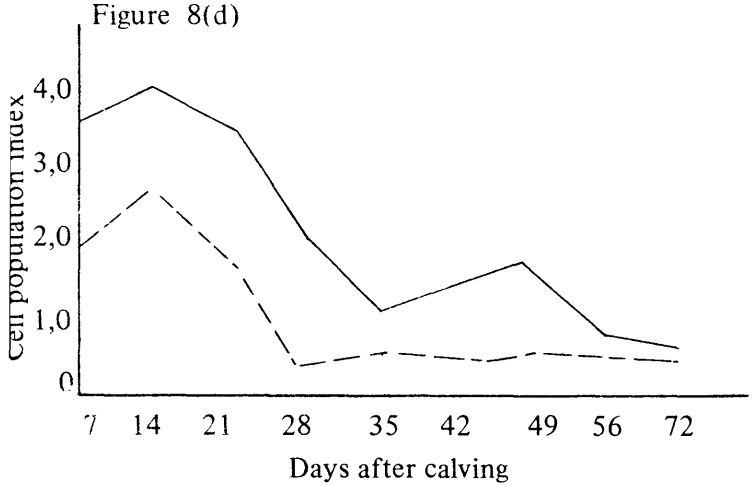
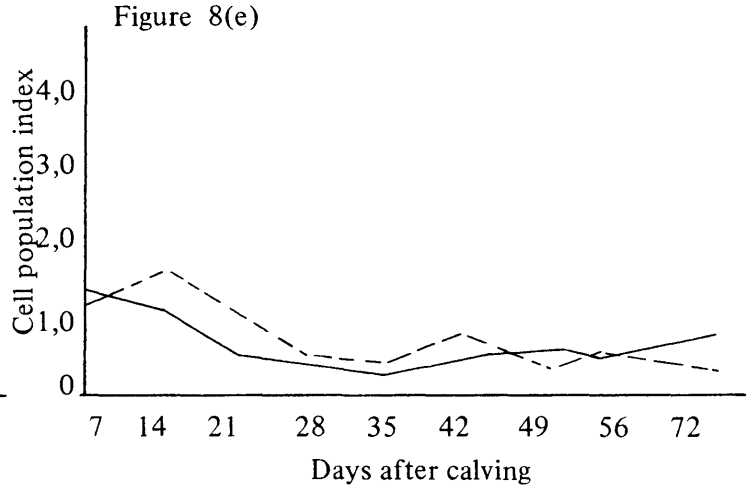


Figure 8(d)



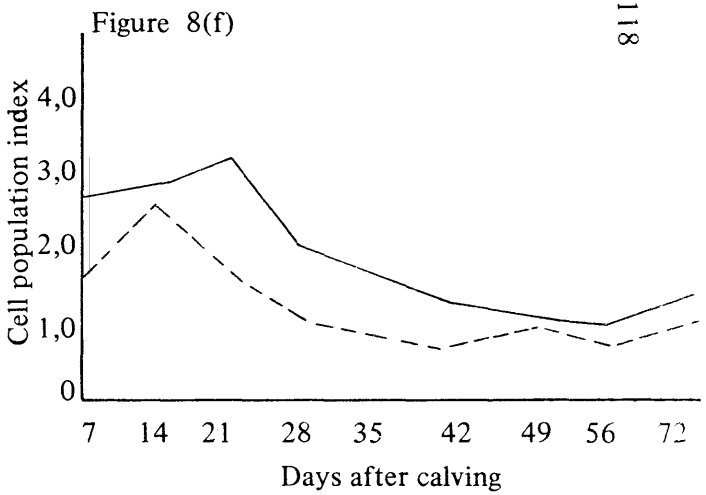
NEUTROPHILES

Figure 8(e)



EOSINOPHYLES

Figure 8(f)



HISTIOCYTES

FIGURE 8: Respective cell populations according to a numerical index of bovine endometrium taken at weekly intervals from 7 to 72 days after calving in cows on a HIGH and a LOW level of feeding respectively.

3.2.4.2 Neutrophiles

The neutrophile population of the endometrium underwent a dramatic change during the puerperium with a sharp difference in intensity between the HIGH and the LOW groups.

At 7 days after calving, there was already a highly significant ($P < 0,01$) increase in neutrophiles in both groups. Their numbers kept on rising until 14 days after calving. This was followed by a significant decline in both groups, reaching the "normal" level at least one week earlier in the LOW than in the HIGH group.

The difference in endometrial neutrophile population between the two groups from 7 days until 56 days after calving, is most apparent according to Figure 8(d). Neutrophile numbers in the LOW group maintained a remarkably constant and low level (possibly the "normal" level) from 28 days after calving for the rest of the entire period, while the neutrophiles in the HIGH group remained at a considerably higher and at a fluctuating level throughout. It is further clear from Figure 8(d) that the neutrophile population in the HIGH group settled down to "normal" level only at 56 days after calving as against 28 days in the LOW group.

Statistically, differences between the two groups were highly significant ($P < 0,01$) at 7 and 28 days after calving and significant ($P < 0,05$) at 21, 42 and 49 days after calving.

3.2.4.3 Plasma cells

The pattern of plasma cell numbers in the endometrium in both the HIGH and the LOW groups showed an initial increase during the first 14 days after calving. (Figure 8(b).) This was followed by a decrease with a temporary rise again by day 49 after calving.

Plasma cell numbers were significantly increased ($P < 0,05$) in the HIGH group between 7 and 28 days after calving, followed by a significant ($P < 0,05$) decrease from days 14, 21 and 28 until days 56 and 72 after calving.

In the LOW group there was a highly significant ($P < 0,01$) decrease in plasma cell numbers between 14 and 37 to 72 days after calving.

Between groups there appeared to be a higher plasma cell population in the HIGH group than in the LOW group from 14 until 49 days after calving although this difference was statistically not significant.

3.2.4.4 Lymphocytes

The overall lymphocyte population of the endometrium followed the pattern of plasma cells rather closely.

In the HIGH group a highly significant increase ($P < 0,01$) in lymphocytes occurred between 7 and 14 days after calving and this was maintained through to day 28 after calving. A further significant increase ($P < 0,05$) was recorded at day 35, followed by a decrease which was significant ($P < 0,05$) at day 72 after calving.

In the LOW group the initial rise was significant ($P < 0,05$) only at day 14, followed by a decrease which was significant ($P < 0,05$) by day 35. This was followed by a temporary rise and then a significant ($P < 0,05$) decline towards day 72.

Between the two groups, the HIGH group appeared to exhibit a more concentrated lymphocyte population than the LOW group from 14 to 42 days after calving. This difference was significant ($P < 0,05$) at day 35.

3.2.4.5 Eosinophiles

The first 14 days after calving was characterised by an elevation in the eosinophile population of the endometrium in both groups. This was followed by a decline until day 35 after calving, after which the eosinophile numbers remained at a constant but mildly fluctuating level until observations were discontinued.

There was a significant ($P < 0,05$) reduction in eosinophiles in the HIGH group between day 7 and day 35 after calving.

In the LOW group, the corresponding reduction was extended and it was significant ($P < 0,05$) from days 7 and 21 to days 28, 35 and 48 and highly significant ($P < 0,01$) from day 14 to days 28, 35, 49, 56 and 72. There was further a significant ($P < 0,05$) reduction from day 14 to day 42.

Between the two groups therefore, the LOW group exhibited a higher eosinophile population in the endometrium during the first 28 days after calving than the HIGH group although this difference was statistically not significant however.

3.2.4.6 Mast cells

The overall mast cell population of the endometrium during the puerperium was somewhat reminiscent of the eosinophile population of the two groups. An initial elevation in mast cell numbers was recorded during the first 14 days after calving, followed by a decline until day 28 after which mast cell numbers fluctuated at this lower level in both groups.

In the HIGH group at 56 days, mast cell numbers were highly significantly ($P < 0,01$) lower and at 72 days significantly ($P < 0,05$) lower than at 7 and at 14 days after calving.

In the LOW group, mast cells numbers were highly significantly ($P < 0,01$) lower at days 28 to 72 than at day 14 after calving, and at days 28, 35, 49 and 72 significantly ($P < 0,05$) lower than at day 21 after calving.

Between the two groups, mast cell numbers appeared to be markedly higher in the LOW than in the HIGH group during the first 21 to 28 days after calving, followed by fluctuations in both groups at a reduced level of mast cells.

Differences between the groups in mast cells, were strikingly reminiscent of differences in eosinophiles, but statistically not significant.

3.2.4.7 Histiocytes

The histiocyte population of the endometrium during the puerperium was reminiscent of the neutrophile population.

At 35, 42 and 72 days after calving, histiocyte numbers were significantly fewer ($P < 0,05$) and at 49 and 56 days highly significantly fewer ($P < 0,01$) than at 7 and 14 days after calving in the HIGH group. At 35 and 42 days after calving in the HIGH group, histiocytes were significantly fewer ($P < 0,05$) and at 49, 56 and 72 days, they were highly significantly fewer ($P < 0,01$) than at 21 days.

At 14, 42 and 56 days in the LOW group, histiocyte numbers were significantly fewer ($P < 0,05$) than at 7 days. At 21 days, there were significantly fewer ($P < 0,05$) and at 28, 35, 42, 49, 56 and 72 days there were highly significantly fewer ($P < 0,01$) histiocytes than at 14 days, while at 42 and 56 days they were significantly fewer ($P < 0,05$) than at 21 days.

Between the two groups, histiocyte activity was higher for the first 42 to 49 day period after calving in the HIGH than in the LOW group according to Figure 8(f). This difference was significant at 7 and at 21 days after calving ($P < 0,05$).

3.2.4.8 Mobile cells in the surface epithelium in the HIGH and LOW feeding groups

The surface epithelium was frequently invaded by migrating cells and neutrophiles, lymphocytes and histiocytes were readily recognized between the columnar epithelial cells. Plasma cells, mast cells and eosinophiles were not noticed in the surface epithelium.

The average number of each cell type was calculated per microscopical field for all biopsy specimens for the two groups from 7 until 72 days after calving.

Figures 9(a) and 9(b) illustrate the numbers of neutrophiles, lymphocytes and histiocytes per microscopical field in the endometrial surface epithelium for the HIGH and the LOW groups respectively.

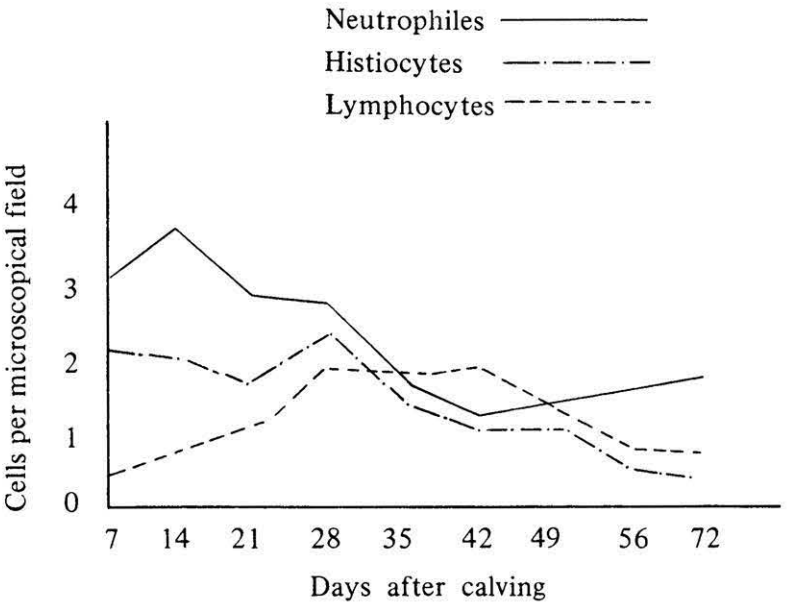
3.2.4.9 Cell types during the follicular and the luteal phases of the cycle

When the cows started cycling again after calving, the mobile cell population of the endometrium was investigated from biopsy specimens taken on the day of estrus and again during the luteal phase between days 12 and 14 of each cycle.

Because of the limited number of cycles exhibited by cows in the HIGH group during the puerperium, a comparison of cell types between the two groups was not possible and it was decided to investigate the occurrence of mobile cells in the two groups combined at estrus and during the luteal phase.

Table 8 presents the numbers of the respective cell types studied, calculated according to the numerical index employed in this investigation, together with the standard deviation for the various cell types for the HIGH and LOW groups together at estrus and during the luteal phase.

(a) HIGH feeding level



(b) LOW Feeding level

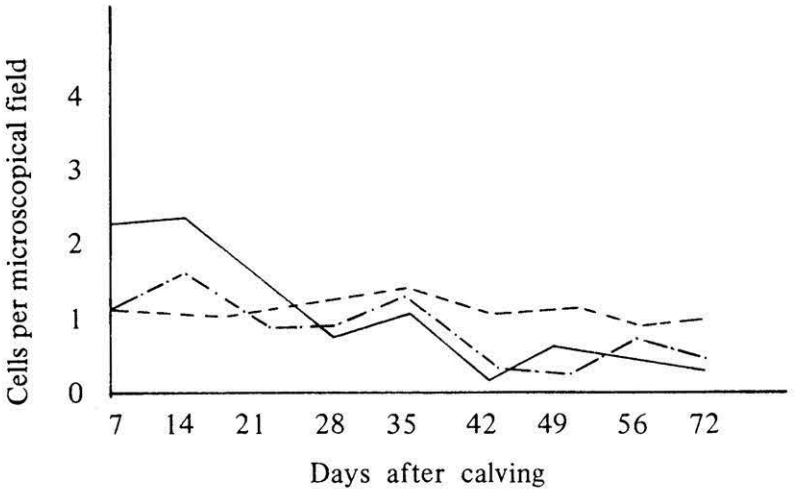


FIGURE 9: The number of neutrophiles, lymphocytes and histiocytes per microscopical field in bovine endometrium epithelium at weekly intervals in cows on high and low feeding levels

TABLE 8: The average numbers of mobile cells in the endometrium, calculated according to a numerical index, together with the standard deviation, during estrus and the luteal phase of the HIGH and LOW groups together

Cell type	Estrus		Luteal phase	
	Number	SD	Number	SD
Neutrophiles	2,030	1,311	1,090	1,162**
Eosinophiles	1,360	0,917	0,960	0,910*
Lymphocytes	2,530	0,969	3,030	1,187*
Plasma cells	2,000	0,938	1,720	1,171
Mast cells	1,830	0,939	0,930	0,930
Histiocytes	1,950	1,008	1,710	1,134**

*P< 0,05

**P< 0,01

3.2.4.10 Special features of the cells in the uterine wall

Further to the cell counts recorded above, certain features were recorded during the examination of microscopical sections which, although they have no direct bearing on the comparative endometrium histology of the two groups, provide valuable information on some features of the cellular distribution of the endometrium.

Localization of mobile cells was a common feature that was recorded regularly in this study. Eosinophiles, lymphocytes, plasma cells and neutrophiles and particularly mast cells, showed a tendency to group together in many sections as well as occurring singly and always with a higher concentration in the stratum compactum than in the stratum spongiosum.

Capillary vessels were often tightly packed with some of these cells, particularly neutrophiles or eosinophiles where no other blood cells could be noticed in the vessel at times. Perivascular distribution of cells were common, particularly where large numbers of a cell type were present in the vessel.

Mast cells were in some sections seen to concentrate around some lymph follicles and in a few sections a single row of mast cells were seen closely packed below the endometrium surface epithelium.

In some cows in either group, and at certain times during the post parturient period between days 10 and 20, sporadic and exceptionally severe increases were encountered in mast cell or in eosinophile numbers, 50 to 100 cells per microscopical field having been counted. These increases were naturally not reflected in the index system employed.

Duration of the increases were of a temporary nature and returned back to the average in periods of 7 to 14 days. Neither could these increases be related in any way to any clinical event in the cows concerned.

The size of mast cells as well as the number of granules concentrated around their nuclei, varied distinctly within sections. In most sections too, detached mast cell granules were observed singly or in groups or spread out over an area giving the impression that mast cells had been ruptured with a loss of granules.

Pseudostratification of the surface epithelium was not seen. It was possible to orientate biopsy specimens to do sectioning at right angles to the epithelium which eliminated illusionary pseudostratification through cutting through an oblique angle.

The attachment of surface epithelium is exceptionally loose. It detaches readily and is sometimes lost in spite of very careful handling of biopsy material.

3.2.4.11 Epithelial cell height and nuclei

The epithelial cell height of the endometrium in each biopsy was recorded as high, medium or low and nuclear appearance was recorded as vesicular or dark.

The percentage of tall cells and vesicular nuclei recorded in the two groups respectively during the 7 to 72 day period after calving, was calculated and this is illustrated in Figures 10(a) and 10(b).

It is clear from Figures 10(a) and 10(b) that vesicular nuclei were associated with an increase in cell height and dark nuclei with a decrease in cell height.

The presence of tall epithelial cells with vesicular nuclei in the endometrium appeared, according to Figures 10(a) and 10(b), to be at a maximum for both groups from 8 to 14 days after calving and then to decline until 35 to 42 days after calving after which an increase seemed to occur again.

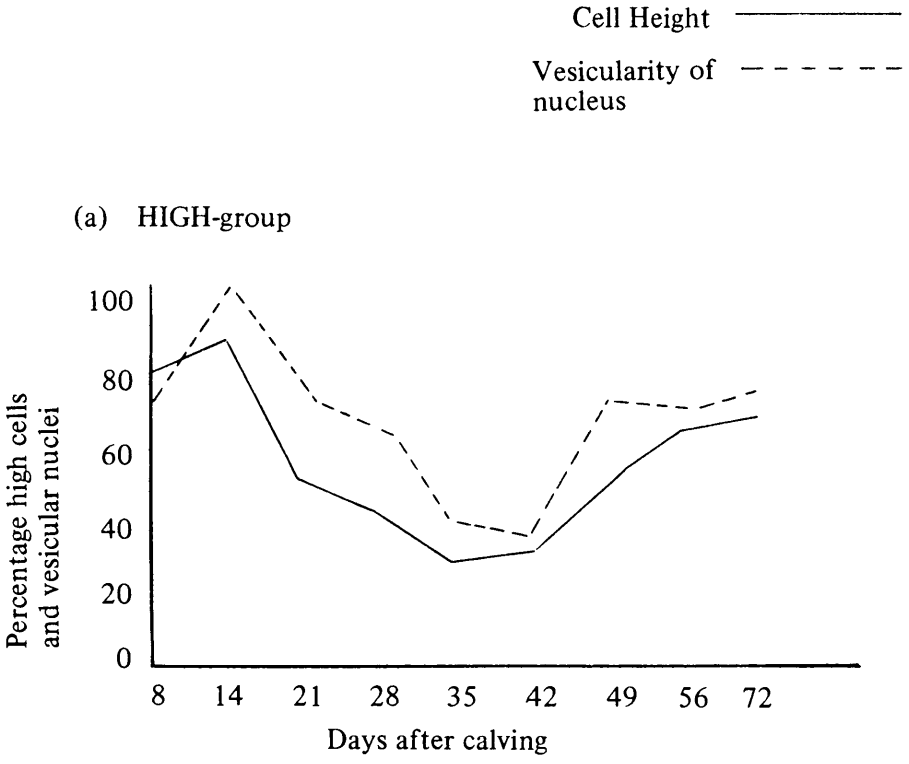


FIGURE 10: Cellular height (solid line) and vesicularity of nuclei in endometrial epithelium during the post calving period

Changes in the epithelial cells seemed to follow the same pattern in both groups, but changes in the HIGH group appeared to be of greater intensity than in the LOW group.

Cell height and vesicularity of the nuclei were also compared at estrus and during the luteal phase for each group. Again, the number of cycles were low for the HIGH group to draw conclusions, but there appeared to be no differences between the two groups. Within the groups though, estrus seemed to be characterised by an increased appearance of vesicular nuclei while no differences in cell height could be observed between estrus and the luteal phase.

It was however a regular feature throughout the examination of biopsy specimens, for variations in cell height and nuclear vesicularity to be recorded in the same section and even in the same microscopical field.

Vesicular as well as pyknotic endometrium surface epithelium cells were recorded in the same microscopical field, while low cells, or cells of medium height could be demonstrated to possess vesicular nuclei, contrary to the general trend illustrated in Figure 10.

This was strongly indicative of an individual rhythm of cells or groups of cells in the endometrium rather than the entire surface epithelium or large portions of it functioning at the same or comparable level at any given stage, particularly as large vesicular nuclei occurred adjacent to dark and pyknotic nuclei.

3.2.4.12 The endometrial glands

Endometrial glands were present in all biopsy specimens and their numbers varied from only a few glands in some specimens, to large numbers of glands in most of the sections.

The cell height and the appearance of glandular nuclei were recorded. Infiltration of mobile cells between the glandular epithelial cells were recorded and the lumina of glands were studied in relation to size and the presence and appearance of debris within the lumen.

These features were compared between the two groups and also at estrus and during the luteal phase between the two groups and within groups.

Variations within groups and even within the same specimen, overshadowed any differences that could be established between groups.

Although the purpose of this investigation was a comparison of the endometrium glandular histology between the two groups, results are nevertheless presented as general histological features of the bovine endometrial glands, much of which is not well recorded in literature.

3.2.4.12.1 Wandering cells

Neutrophiles and lymphocytes were the only mobile cells that were recorded to migrate through the glandular epithelium into the lumen. Migration of these cells in the glandular epithelium coincided with migration into the surface epithelium.

3.2.4.12.2 Glandular debris

Debris was present in ever varying numbers of glandular lumina in every section. Debris varied in appearance from homogenous material to cellular parts in which pyknotic nuclei and degenerated cells could be distinguished. In many cases nuclei were radially arranged in glandular lumina forming a complete circle which left the impression that the entire epithelium of the gland was rejected into the lumen.

Mobile cells were often recognized in the glandular lumina, particularly lymphocytes and neutrophiles but also plasma cells, mast cells and eosinophiles. It was apparently incidental that the latter three cell types were not seen to migrate through the epithelial cells.

It was frequently observed in the same section that glandular lumina would vary widely in appearance. Some glands would have a narrow and vacant lumen, while in others it would be wide with or without a fluid content or with cellular or degenerated debris.

Variations in the same section and often in adjacent glands in the same microscopical field far exceeded any variations between different stages in the process of involution or between different cows or even between the glands at estrus and during the luteal phase of the cycle.

3.2.4.12.3 Glandular nuclei

In most of the sections, the nuclei of glandular epithelial cells varied from large vesicular nuclei with fine chromatin granules to small and elongated dark staining pyknotic nuclei.

It was a typical and a regular feature of the nuclei in the glands in the stratum compactum and towards the surface to exhibit nuclei of the large vesicular type, while pyknotic nuclei were a feature of the glandular end portions in the deeper layers of the endometrium.

It was however by no means unusual to find vesicular as well as pyknotic nuclei in a cross section of the same gland.

Debris, or cellular matter in the lumina of glands, was also not restricted to one or other type or appearance of glandular nuclei and was recorded as often with vesicular as with pyknotic nuclei.

3.2.4.12.4 Glandular cell height

Glandular cell height varied from a low cuboidal to a high cylindrical epithelium with a vacuolated appearance.

Variations in cell height within specimens overshadowed variation between specimens and no clear differences could be demonstrated between different stages of uterine involution or between the different stages of the cycle.

3.2.4.12.5 Glandular lumina

The lumina of endometrial glands varied within the same section and between sections taken of the same cow at the same stage after parturition as much as at different stages after parturition or between the different stages of the cycle. Glandular lumina were recorded to be wide and empty or with a content of debris, all within the same section, as well as narrow or practically absent in adjacent glandular sections.

3.2.4.13 Lymph follicles in the endometrium

Lymph follicles were recorded in approximately one half of the cows and to the same extent in both groups.

Lymph follicles were not encountered before 15 days after calving at the earliest. In most cows, lymph follicles developed in the endometrium between 15 and 30 days after calving, and they disappeared again after a presence of a few weeks.

The development of lymph follicles was not necessarily incidental to any post parturient complication. They were recorded in cows that went through completely normal and uncomplicated uterine involution in the LOW group, as often as in the cows of the HIGH group where involution was complicated and delayed.

Lymph follicles were associated with an increased population of lymphocytes in the endometrium and a simultaneous decrease in neutrophiles, while the plasma cell, histiocyte and mast cell population of the endometrium appeared to be unaltered.

3.2.4.14 Subepithelial haemorrhage

Subepithelial extravasation of blood was recorded immediately below the surface epithelium as well as deeper in the stroma of the endometrium in approximately one half of all biopsies, and involving all cows.

The extent of the haemorrhages varied from mild extravasation of isolated groups of erythrocytes to peticheal haemorrhages of various sizes.

The presence or extent of free blood in the endometrium could not be correlated with any clinical event in the animal concerned. It was concluded that trauma with the biopsy instrument was responsible for the haemorrhages.

All haemorrhages were of such localized distribution that examination of specimens was unaffected.

3.2.5 Discussion

Certain morphological characteristics of bodily cells are indicative of their functional stages.

Increased secretory activity of epithelial cells is responsible for an increased cell height and an accumulation of secretion which can be studied by means of light microscopy.

Nuclear chromatin aggregation which gives the nucleus a vesicular or pyknotic appearance, indicates secretory activity or regeneration. Trump & Ericsson (1965) sum up the present state of our knowledge on chromatin aggregation and distribution.

It is a rapid process and is among the earliest responses of the cell to injury. The process might be reversable in the early stages. As necrosis proceeds, aggregated chromatin presents a rarified appearance and then disappears as result of attack by DNase, and the nucleus exhibits classical karyolysis.

The ultra-structural equivalent of pyknosis has however not been determined. Chromatin fibrils propably aggregate as described above but it is apparent that to obtain a pyknotic nucleus, which appears to contain a normal amount of DNA, the nucleus would have to decrease in volume, possibly as a result of water loss before DNase attack on chromatin.

The function of the various mobile cell types has been discussed and changes in their presence in the endometrium can be interpreted in terms of functional or reactional changes in the tissue.

Cyclic, as well as involutionary changes in the endometrium were characterised by considerable individual differences which made a clear distinction between physiological and pathological manifestation impossible in many cases. This was also clear from results obtained in clinical observation on the genitalia.

It is further clear from this investigation, that no clinical or histological examination of the genitalia at any given stage during the pueperium or during the cycle, would have given a reliable indication of its state of normality or its functional efficiency. It was relatively easy however to distinguish normal from abnormal states of the uterus or ovaries in terms of clinical events however and it is suggested that certain physiological and pathological extremes are quite closely related. Differences in mobile cell population of the endometrium, might demonstrate mere differences in the state of "alert" or "alarm" between cows or groups as well as true abnormalities.

Skjerven (1956) likewise recorded that a small or marked variation from animal to animal was present for all cell types studied by him, even under

apparently similar biological conditions. He mentions however that a few biopsies differed markedly from others in regard to their high content of individual cell types but he failed to find any relation between a high number of one cell type and a high number of another cell type. Nor could he observe any definite conditions in the animal which explains the variation in cell types. He concluded that it seemed reasonable that biopsies with maximal cell numbers, should be regarded as extreme variates in a physiological variation.

The reaction of the endometrium to bacterial invasion has been recorded by several investigators.

Dawson (1950) summarized the early results of several workers and reported that bacteria are recorded in moderately high density in the normal bovine uterus, while Kampelmacher (1954) reported that the uterus of the normal cow is free from bacteria and able to destroy introduced organisms in 48 hours, the bactericidal power of the uterus being attached to the living tissue.

In cows with endometritis, he reported that bacteria could still be demonstrated after 5 days.

It was reported by Gunter, Collins, Owen & Sorenson, Scales & Alford (1955) that 39 per cent of normal cows in a Hereford herd had sterile uteri while only 5 per cent of repeat breeding cows had sterile uteri. In a study to determine whether micro-organisms were responsible for the incidence of embryonic death at 16 days post-breeding, Hawk, Kiddy, Wilson, Esposito & Winter (1958) reported that microbial infection accounted for few of the embryonic deaths in clinically normal repeat breeding cows.

Sagartz & Hardenbrook (1971) studied the bacterial population of bovine uteri in relation to histopathological lesions and reported that lesions in the endometrium (90 per cent) exceeded yield of bacteria (62 per cent). They concluded that factors other than bacteria diminish fertility.

In this investigation it was pointed out in Table 4(a) that the high level of feeding contributed to lowered fertility by means of a mechanism in which endocrine imbalances might operate.

In addition to this, it was shown that the high feeding level was responsible for a delayed rate of uterine regression which induced favourable conditions for bacterial proliferation.

Elliot, McMahon, Gier & Marion (1968) isolated various known pathogenic organisms from the uteri of post partum cows and recorded 93 per cent of uteri infected within 15 days after calving and 9 per cent between 46 and 60 days. Kudlac (1971) recorded that the bacterial content of the post partum uterus rose up to the 11th day and then declined and that at four weeks after parturition, hardly any bacteria could be demonstrated.

He further states that in the aetiology of endometritis, interference with motility, lowered contractile power and the ability of the uterus to clean itself are more important than actual bacterial infection. Substances that therefore benefit the myometrium are more useful in the treatment of puerperial diseases than the use of antibiotics.

The rate of uterine regression is evidently of prime importance in puerperal endometritis.

Gier & Marion (1968) recorded involutionary changes in the uterus to be final at 40 to 50 days after calving while Buch, Tyler & Casida (1955) recorded an average interval of 47 days with a difference between primaparous cows (42 days) and pluriparous cows (50 days). In abnormal calvings they recorded a longer interval.

Heat stress was also recorded to increase the time taken from parturition to completion of uterine involution by Marion, Norwood & Gier (1968). They confirmed the role of parity and reported that although palpable regression might be complete by 40 days, histo-morphological changes proceed until 50 days after calving.

Casida (1968) reported that suckling decreased the endometrial glandular mass and lymphocytes while interval since calving increased polymorphonuclear leucocytes and lessened the severity of experimentally induced endometritis. He agreed that the large physical size of the uterus after calving, the sloughing of cotyledons and the high counts of infiltrating cells were related to lowered fertility.

Tennant & Peddicord (1968) confirmed the average time taken to completion of uterine regression of 42 to 50 days on palpation and that abnormal calvings, twins, retained placentae, prolapse, dead calves and abortions resulted in a delay in involution. Fertility appeared to be unaffected by the state of involution of the normal uterus but was significantly reduced by metritis or by a delay in involution.

Perkins and Kidder (1963) studied the role of uterine involution in beef cattle by internal palpation and reported that intervals from calving to completion of involution was 36,5 days in Herefords and 38,7 days in Angus cows. They also reported that conception rate was unaffected by the involutionary state of the uterus at the time of breeding.

In terms of this investigation, the interval from calving to completion of uterine involution varied significantly between the two groups and also between palpation and histo-pathological examination as shown in Table 5 and Figures 8(a)–

8(f). On histo-pathological examination the end point of involution was vague as shown by the interrelationship of the various cell types in the endometrium in which profound differences were demonstrated between the two groups.

It is suggested that on palpation, the musculature of the uterus is responsible for uterine size and therefore palpable. On histological examination again, the endometrium cellular composition is evaluated and this plays a more important role in the re-establishment of fertility after calving. Studies of uterine involution by palpation only might therefore have a limited value.

Summarily, then, it is well recorded that retarded uterine involution predisposes to endometritis with bacterial proliferation in the uterus and reduced fertility. The normal bovine uterus responds favourably to bacterial invasion but any factor that retards the expulsion or absorption of lochia or uterine debris, promotes bacterial proliferation and endometritis.

In this investigation these conditions were perfectly met in the cows on the high level of feeding and would indeed have been responsible for a herd fertility problem. An increase in uterine size over the entire period of regression and a very considerable delay in involution followed prolonged parturition and a high incidence of dystokia in this group.

Histologically, these complications were clearly reflected in the endometrium as illustrated in Figure 8.

The neutrophile infiltration in the HIGH group in Figure 8(a) suggests a pathological condition and this is substantiated by the increased histiocyte activity in this group. (Figure 8(f)). These histological features were related to the clinical findings that were recorded for this group.

In the LOW group on clinical examination, no problems were recorded. Histologically however, the pattern of neutrophile and histiocyte activity followed that of the HIGH group.

This means that the differences were only in degree and suggests that cell numbers were within physiological limits in one group but above physiological limits in the other group.

The patterns of mast cell and of eosinophile invasion of the endometrium bear a close resemblance according to Figures 8(c) and 8(e). An initial rise during the first 14 days is followed by a reduction in numbers and a somewhat fluctuating level of each cell type in the subsequent period.

This corresponds to the occurrence of neutrophiles and of histiocytes in the endometrium during the post partum period although these cell types were recorded in considerably larger numbers particularly in the HIGH group.

An inverse relationship however exists between the HIGH and the LOW groups in mast cells and eosinophiles on the one hand and neutrophiles and histiocytes on the other. Although differences were shown to be statistically not significant, a tendency exists for mast cell and eosinophile numbers to be higher in the LOW group while neutrophile and histiocyte numbers were significantly higher in the HIGH group during the first 28 days of the post partum period, which was easy to explain in terms of bacterial invasion and the removal of cellular debris in the HIGH group.

One is tempted to interpret the relative increase in eosinophiles and mast cells in terms of the association which is known to exist between eosinophiles and the adrenal, and the role of mast cells in smooth muscle contraction, capillary permeability and the establishment of tissue homeostasis as explained earlier on in this dissertation.

Although this is speculative, it fits in well with the clinical observations that were reported.

The alterations in the numbers of lymphocytes and plasma cells show a remarkable resemblence. An initial rise was recorded in each of these two cells types during the first 14 days after parturition. This was followed by a decline in numbers of both cell types in the LOW group, particularly at day 35, while in the HIGH group the elevated level of both cell types appeared evident until days 42 to 49.

A higher level of lymphocyte and plasma cells in the endometrium of cows in the HIGH group, was evident particularly during the period from 21 to 42 days after parturition.

It is also clear that the lymphocyte and plasma cell populations of the endometrium during the post partum period followed an entirely different pattern to that of the other four cell types studied.

Again, the significance that can be attached to the alterations in the lymphocyte and plasma cell numbers recorded in this study in the endometrium between the two groups, is a matter of conjecture based on the role of these cells in tissue immunology.

Differences of the cellular population in the deeper layers of the endometrium were reflected in cellular migration through the endometrial and glandular epithelia. The reason for this migration is not clear and might be incidental to the respective cell populations of the deeper layers of the uterine wall.

Again, the increased activity of neutrophiles and histiocytes in the HIGH group as against the LOW group indicates an increased state of "stress" or "alarm" in this group while a clear difference exists in lymphocytic levels. Even in in cellular height and nuclear vesicularity of the endometrial epithelium, more abrupt changes are apparent in the HIGH group. It was indicated that these changes reflect changes in cellular activity.

Whilst no cyclic differences could be demonstrated between groups, the defensive mechanism of the endometrium at estrus against bacterial invasion is clear from significant to highly significant increases in neutrophiles, histiocytes and lymphocytes.

A significant increase was demonstrated for eosinophiles at estrus, a cell type of which the function in the endometrium is not clear.

Individual variations in endometrial glands confirm the individual rhythm recorded for glandular activity as well as the fact that the superficial glands are more active than the deep glands.

The causes or the function of the cellular content of the endometrial glands is obscure and might be incidental to glandular regeneration.

TABLE 9 – Blood serum values with standard duration (in parenthesis) of Friesland cows on a high and a low level of feeding before the onset of treatment (month 1) and again 9 and 24 months later (month 9 and month 24 respectively)

(a) HIGH group Serum value	Month 1		Month 9		Month 1		Month 24		Month 9		Month 24		Normal***
N %	1,165	(0,089**	1,297	(0,070)	1,165	(0,089)	1,235	(0,083)	1,297	(0,070)	1,235	(0,083)	1,15 – 1,26
Protein %	7,289	(0,516)**	8,113	(0,368)	7,289	(0,516)	7,730	(0,506)	8,113	(0,368)	7,730	(0,506)	7,16 – 7,85
Lipids mg %	114,200	(16,819)	103,931	(12,311)	114,200	(16,819)	108,653	(11,484)	103,931	(12,311)	108,635	(11,484)	
Na^+ mEq/l	111,033	(10,052)	105,404	(8,733)	111,033	(10,052)	102,866	(8,882)	105,404	(8,733)	102,866	(8,882)	135 – 143
K^+ mEq/l	4,470	(0,504)	4,610	(0,539)	4,470	(0,504)	4,812	(0,808)	4,610	(0,539)	4,812	(0,808)	3,9 – 5,6
Ca^{2+} mEq/l	4,272	(0,521)	4,178	(0,238)	4,272	(0,521)	4,330	(0,346)	4,178	(0,238)	4,330	(0,346)	4,14 – 5,69
Mg^{2+} mEq/l	3,405	(0,109)*	3,179	(0,244)	3,405	(0,109)	3,406	(0,187)	3,179	(0,244)*	3,406	(0,187)	1,48 – 2,63
PmEq/l	3,073	(0,273)**	3,647	(0,544)	3,073	(0,273)	3,315	(0,320)	3,647	(0,544)	3,315	(0,320)	3,49 – 6,97

** P< 0,01
* P< 0,05

(b) LOW group Serum	Month 1		Month 9		Month 1		Month 24		Month 9		Month 24		Normal***
N %	1,194	(0,054)	1,214	(0,094)	1,194	(0,054)	1,214	(0,070)	1,124	(0,094)	1,214	(0,070)	1,15 – 1,26
Protein %	7,465	(0,336)	7,589	(0,583)	7,465	(0,336)	7,591	(0,403)	7,589	(0,583)	7,591	(0,403)	7,16 – 7,85
Lipids mg %	113,234	(21,053)	100,356	(17,989)	113,234	(21,053)	114,512	(20,398)	100,356	(17,989)*	114,512	(20,398)	
Na^+ mEq/l	110,954	(9,412)	107,520	(5,339)	110,954	(9,412)	106,933	(14,206)	107,520	(5,339)	106,933	(14,206)	135 – 143
K^+ mEq/l	4,559	(0,322)	4,826	(0,434)	4,559	(0,322)	4,724	(0,737)	4,826	(0,434)	4,724	(0,737)	3,9 – 5,6
Ca^{2+} mEq/l	4,366	(0,374)	4,295	(0,531)	4,366	(0,374)	4,432	(0,494)	4,295	(0,531)	4,432	(0,494)	4,14 – 5,69
Mg^{2+} mEq/l	3,381	(0,209)**	2,991	(0,248)	3,381	(0,209)	3,403	(0,099)	2,991	(0,248)**	3,403	(0,099)	1,48 – 2,63
PmEq/l	3,047	(0,296)**	3,405	(0,324)	3,047	(0,296)	3,176	(0,608)	3,405	(0,324)	3,176	(0,608)	3,49 – 6,97

** P <0,01
* P <0,05

***Doxey (1971)

Differences between the two groups were not statistically significant

TABLE 10 – **Clinical pathological values of blood serum specimens of cows maintained on a high and a low level of feeding respectively during 24 months (S.D. in parenthesis)**

Average Serum Value		HIGH group		LOW group		Normal*
Haematocrit	%	37,420	(3,628)	36,900	(2,095)	24–40
White cell count		9757,140	(1444,078)	9618,180	(2462,015)	4000–10000
Neutrophiles	%	46,140	(8,766)	44,360	(11,119)	15–45
Lymphocytes	%	49,280	(9,034)	50,090	(18,035)	45–75
Monocytes	%	2,420	(1,412)	1,720	(0,970)	2–7
Eosinophiles	%	2,140	(1,887)	1,000	(1,276)	0–20
Basophiles	%	0	(0)	0	(0)	0–2
SGO-T	i.u.	41,030	(8,753)	42,680	(10,645)	20–62
SGP-T	i.u.	7,250	(4,263)	7,470	(2,083)	3–12
Cholesterol	mg/100 ml	192,000	(47,503)	230,270	(57,959)	100
Thyroxine	mg/100 ml	3,500	(0,800)	3,310	(0,723)	3–5
Total serum protein	g/100 ml	7,580	(0,594)	7,790	(0,515)	7,16–7,85

*Doxey (1971)

Differences between the two groups were not statistically significant

3.3 Blood serum analysis, clinical pathological values and liver mineral content

3.3.1 *Results of blood serum analysis* at various stages during the trial, together with the standard deviation are summarized in Tables 9(a) and 9(b) respectively for the two groups. An average for individual cows was first calculated from the three specimens collected each time. Averages before the onset of treatment are referred to as month 1. Values obtained 9 and 24 months after the onset of the treatment, are referred to as month 9 and month 24 respectively.

3.3.2 *Results of the clinical pathological analysis* on serum specimens of the two groups at the end of the trial, are set out in Table 10.

3.3.3 *The mineral content of liver specimens* of those cows in the two groups that were slaughtered at the end of the trial, are summarized in Table 11.

According to Tables 9(a) and 9(b) certain significant and highly significant differences were recorded in serum values within the two groups between month 1 and month 9 of the trial and also between month 9 and month 24. At month 9, all cows were dry and received no additional rations for production. In both groups, serum P was higher and Mg lower during this period, while serum N and proteins were higher in the HIGH group and serum lipids lower in the LOW group.

Average serum values were somewhat lower for Na and higher for lipids in both groups in this study than the normal values reported for the bovine.

The reason for, or the significance of these differences, are not clear and must remain a matter for speculation.

Of great importance for the purposes of this study however, is the fact that the very considerable difference in nutritional level between the two groups did not induce any identifiable or abnormally high or low serum value according to Table 9 in any cow in either of the two groups, neither could any significant difference be established between the two treatment groups.

Likewise, according to Tables 10 and 11, all the values fall within the normal limits that are on record for bovines. No significant differences exist between the HIGH and the LOW groups in clinical pathological values (Table 10),

TABLE 11 – **Mineral content of the livers of cows kept on a high and a low level of feeding respectively (on a wet basis).**

Cow No.	Cu	Zu	Co	Mn	Fe	Mg
HIGH group						
5	56,9	93	18,6	5,2	122	173
7	66,2	102	20,0	6,1	118	200
9	67,3	92	18,2	6,2	126	180
12	35,6	83	11,2	4,7	109	172
14	30,8	102	15,6	7,7	131	200
18	34,3	182	17,1	6,7	122	224
24	71,0	113	17,9	7,6	142	176
25	71,2	124	17,7	7,2	124	205
30	101,6	185	14,0	7,2	212	206
32	61,3	149	19,4	4,7	66	174
Mean	59,6*	122,5	16,9	6,3	127,2	191,0
LOW group						
1	20,3	93	18,9	6,0	54	174
6	61,2	252	19,2	6,1	66	181
8	42,3	106	18,9	10	209	211
10	23,8	48	18,4	5,5	93	218
20	34,2	124	15,7	6,6	131	183
27	53,9	103	17,1	6,1	78	168
29	41,4	102	19,2	4,2	74	156
Mean	39,5	118,2	18,2	6,3	100,7	184,4
Normal**	49	158	19	8,1	149	208

*P < 0,05
**According to data collected by the Nutritional Section, Veterinary Research Inst., Onderstepoort on normal animals

neither do any significant differences exist between the two feeding levels in liver mineral values obtained except for Cu which was significantly ($P < 0,05$) higher in the group on the high level of feeding.

Although elaboration on the mineral and trace mineral metabolism of the bovine does not fall within the scope of this dissertation, it is of interest that Sourkes (1970) reports that more copper accumulates in the livers of animals fed 7% dietary protein than 14%.

Kay (1970) however explained that in ruminants it was not uncommon to find large differences in the nature of the population of the rumen micro-organisms and that, if these differences were extended to organisms responsible for producing sulphide, this might have profound effects on the metabolism of trace elements and in particular might account for between – animal differences in the apparent availability and utilization of Cu.

For the purposes of this dissertation however, it is clear that in all the bodily constituents that were estimated and reported above, no excesses or deficiencies could be identified in terms of the present state of our knowledge, neither did any animal in either group, on the basis of the clinical pathological findings, appear to be affected in any of the systems concerned.

A *locus minoris resistentiae* is suggested for each animal in which various and individually different threshold values operate and it appears that the nutritional level plays an important role in the breakdown of these loci. Mills & Chesters (1970) support this view. They believe that many of the discrepancies apparent in statements concerning functional roles of trace minerals for example, arise, apart from inadequate experimental control, from a modification of pathways due to age, duration or severity of depletion of tissue reserves, abnormal intakes, elevated temperatures or forced starvation, all of which lend to a modification of metabolic processes. This, in certain types of animals, is tantamount to termination of productive life-span.

3.4 Mortality and pathological conditions

Mortality and clinical or post mortal abnormalities were restricted entirely to the HIGH group while the LOW group was entirely free from disease throughout, inclusive of post parturient metritis, laminitis or athritis.

The various conditions that were recorded in each of the cows in the HIGH group, together with a summary of the incidence of post parturient metritis, laminitis and arthritis, are presented in Table 12.

Each of the conditions mentioned above has frequently been recorded in cattle.

Fatty degeneration of the liver or, more correctly according to Jubb & Kennedy (1970), hepatic lipidosis, is a common sequel to ketosis in dairy cows. Conditions were favourable in this study for the development of ketosis. The response to treatment in the two cases that were diagnosed was poor, particularly as symptoms were recognised and treated at an early stage.

Adhesions over the uterus and ovaries are common in cattle following metritis, infection spreading via the Fallopian tubes or directly through the endometrium in cases of severe and acute metritis. In these cases crepitation and swelling of the uterine wall are palpable per rectum and are followed by posterior peritonitis.

Permanent sterility follows on uterine and ovarian adhesions, usually with cyst formation on one or both ovaries.

Pleurisy and peritonitis in cattle are usually regarded as sequelae to pneumonia and penetration of a viscus membrane, especially the reticulum or uterus (Jubb & Kennedy, 1970).

Numerous cases have been experienced to have proceeded subclinically even where animals were under close observation eg. intensive dairy units as well as in this investigation.

On examination at slaughtering, fibrous adhesions are then encountered abdominally or between the lungs and pleurae. Considering possible causes of pleuritis, it is not clear how the parietal surfaces of the lung in bovines become affected with pneumonia in the absence of clinical signs of broncho-pneumonia.

In the absence of any traumatic penetration in the abdomen again, it can only be surmized that small and subclinical ulcerations in the digestive tract might play a role in the bacterial infection of the peritoneum in bovines. Reports are however not available on the aetiology of this condition in bovines.

TABLE 12 – **Post parturient complications, laminitis and arthritis recorded in cows on a high feeding level together with clinical symptoms and post mortal lesions at slaughtering**

Cow No.	Post parturient complications	Laminitis	Arthritis	Treatment	Clinical symptoms, post mortal lesions
2	Moderate metritis	Severe	Severe in carpal, tarsal and stifle joints	Antibiotics, intensive symptomatic treatment	Died from extensive fatty degeneration of liver 55 days after calving following ketosis.
5	Severe, vaginal prolapse	Severe	Absent	Uterine and systemic antibiotics	Adhesions around uterus and ovaries Localized chronic pleurisy and peritonitis
7	Severe metritis	Mild	Absent	None	Localized chronic pleurisy and peritonitis. Fibrous adhesions and depressions over extensive areas of surface of liver. Numerous small lesions of liver telangiectasis.
9	Mild metritis	Absent	Absent	None	Localized chronic pleurisy and peritonitis. Fibrous adhesions and depressions over liver.
12	None	Severe in both hind feet, mild in front feet	Severe in both tarsal joints	None	No lesions recorded
14	None	Severe in both hind feet	Both tarsal joints	None	No lesions recorded.

TABLE 12 – (Continued)

Cow	Post parturient	Laminitis	Arthritis	Treatment	Clinical symptoms, post mortal lesions
17	Severe metritis	Severe in hind feet	Severe in hock and stifle joints	Intensive uterine and systemic treatment	Died from fatty degeneration of liver. Minerlization of myocard, following ketosis with necrosis and fibrosis.
18	None	Severe both front feet	Severe in front fetlocks and carpal joints	Antibiotics and corticosteroids	Extensive diffuse grey areas over both kidneys. Histopathologically interstitial nephritis Proliferative endocarditis. Osseous changes and calcareous deposits on carpal and metacarpal bones.
24	Severe metritis	Severe in both hind feet	Severe in both hind fetlocks	None	No lesions recorded.
25	Severe metritis	Severe in both hind feet	Severe in both hind fetlocks	Antibiotic treatment for metritis	No lesions recorded in any organ. Fatty necrosis 12 cm in diameter in omental fat.
30	Chronic metritis after caesarian	Severe in both hind feet	Absent	Intensive and prolonged antibiotics	Fatty necorsis around rectum, chronic pleurisy and peritonitis.
32	None	Severe laminitis front and back feet	Absent	None	No lesions recorded.

A moderately high incidence of pleurisy and peritonitis was recorded in this investigation. If this is taken into account together with impressions gained over many years of clinical experience with cattle, the extent to which pleurisy and peritonitis of unknown origin occur in bovines, is emphasized, although the aetiology of these conditions are very poorly recorded.

It is an accepted fact, well supported by the records of local health authorities at abattoirs, that lesions of chronic pleurisy and peritonitis in bovines, characterized by fibrous adhesions of the serous membranes, are frequently encountered in carcasses at slaughtering, particularly in old animals from intensive farming units. These lesions are rarely seen in young animals or animals derived from extensive systems.

Clinicians engaged in sterility work in bovines agree that fibrous adhesions involving the genitalia are responsible for permanent sterility and it has been motivated that the condition is essentially restricted to intensive units.

Whilst in individual cases, the aetiology of these conditions is explained in terms of "bacterial invasion or infection" no explanation is available in any reports for its higher frequency in intensive units. Admittedly a higher concentration of infective organisms prevail with increased population density, but susceptibility to these infections remains unexplained.

Chilling, exhaustion, old age and intercurrent diseases are predisposing factors according to Jubb & Kennedy (1970) and they conclude that even when the cause is ascertainable, the pathogenesis can seldom be stated with clarity except in those few infections which are consistently virulent.

Two cows in this study had recovered from peritonitis involving the liver and thus left fibrous adhesions and depressions on the surface of the liver, which, together with other peritoneal and pleural fibrous lesions as well as the recorded cases of telangiectasis were not regarded to have had any clinical significance at the time of slaughtering.

One cow had nephritis and both kidneys were affected with extensive but diffuse lesions. According to Jubb & Kennedy (1970) nephritis is of hematogenous origen and usually a complication of a systemic infection. The same cow had severe

arthritis, periostitis and laminitis as well as proliferative endocarditis, the latter condition being considered to be of haematogenous origin as well, and in which the inflammatory condition in the joints might have been a primary lesion.

Mineralization of the mycocard, which was present in one of the cows that died from liver degeneration, is a sequel to myocardial degeneration or necrosis which is known to be of toxic or infectious origin in herbivorous animals (Jubb & Kennedy 1970).

Uncertainty exists on the pathogenesis of abdominal fat necrosis in cattle, two cases of which were recorded in this study.

It is an incidental finding in cattle according to Jubb & Kennedy (1970) and frequently a progressive condition and therefore potentially fatal. They also report that abdominal fat necrosis is not an uncommon condition in cattle in the Channel Islands while Williams, Tyler & Papp (1969) reported it as a herd problem in cattle in Georgia.

They provide evidence that the condition might be dietary in origin and they also quote reports that it might result as a side effect to pyrexia associated with some other primary condition such as peritonitis, enteritis or allergies.

One report (Wilkinson, Stuedemann, Williams, Jones, Dawson and Jackson 1972) suggested that fat necrosis was associated with plant nutrient effects on the chemical composition of fescue. A higher incidence of fat necrosis was recorded in cows grazing on broiler littered fescue that fescue fertilized with ammonium nitrate.

Whether circumstances prevailing in the two cases recorded in this study had any bearing on the development of fat necrosis, is a matter of conjecture. Both cows were from the HIGH group and both suffered severely from laminitis while one of them also had metritis following a ruptured uterus and a caesarian operation. In this latter case, there is little doubt that intestinal obstruction would eventually have been caused by the necrosis which surrounded the rectum.

Summarily therefore, several seemingly divergent conditions were recorded in cows all in the HIGH group. As a possible connection between them, one is inclined to single out the high incidence of post puerperal metritis and laminitis as primary sites of bacterial invasion of cows in this group.

Evidence has however been submitted that the post parturient bovine uterus is relatively resistant to bacterial invasion. In addition, extensive antibiotic treatment was administered, and laminitis is basically an aseptic inflammatory condition.

The question therefore remains whether cows in the HIGH group exhibited a lower threshold against the maladies recorded.

Whilst a common mechanism in the cause of these conditions then remains unidentified, there is little doubt about its existence. These conditions, albeit of seemingly unrelated origen, occurred only in the group on the high level of feeding and was responsible for a most severe reduction in productive life span of the cows in this group, a subject that will be elaborated on further in the discussion on this dissertation.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Summary

The overall result of this investigation was manifested as a number of widely divergent abnormalities. If these conditions are not constantly viewed in terms of the growth and sexual maturation of young heifers and the subsequent effect of a high level of feeding on their performance, the purpose behind this investigation will appear nebulous.

The most important results of this trial are now summarized in chronological sequence as they were presented in the text. It is clear from this investigation that individual animals of higher fertility could be distinguished from their counterparts of lower fertility on the record of their ovarian activity. After the animals of lower fertility were eliminated from the group, the productive life-span and reproductive performance of a further group of them were seriously impaired through over-feeding which exerted an effect in a number of different ways.

4.1 Ovulatory failure and cycle length

A remarkably high incidence of ovulatory failure was recorded in a group of Friesland heifers after the onset of cyclic activity. Delayed ovulation, anovulation and normal ovulation alternated without any fixed pattern in almost every heifer with a gradual tendency towards improvement after the first four cycles.

Only two out of 30 heifers exhibited normal ovulations at all cycles (6 and 7 respectively) from the onset of cyclic activity until conception.

Four out of 30 heifers were sterile, one of which never ovulated for seven consecutive cycles before going into anestrus for 261 days with cystic degeneration of the ovaries. Only 18 heifers (56%) exhibited normal ovulations in more than one half of the total number of estrus periods recorded.

Twice weekly palpations of the genitalia during every cycle and daily palpation at estrus and until ovulation had occurred or regression of the follicle could be established, were required to determine the ovarian activity and ovulatory per-

formance of individual heifers. A single examination cannot be regarded of any value in functional fertility diagnosis.

Pronounced individual differences were apparent between individual animals right from the onset of cyclic activity and serious functional disturbances were recorded in certain individuals as early as the first two cycles after the onset of cyclic activity vis. uterine enlargement (2 out of 30 heifers), spontaneous ovarian haemorrhage followed by adhesions in which the ovary became permanently embedded (2 out 30 heifers), cystic enlargement of the ovaries as well as a high incidence of delayed ovulation and anovulation mentioned above.

Embedding of one ovary in adhesions did not appear to affect the function of the other ovary adversely, neither was cyclic activity interefered with in any way.

Heifers of low fertility could be identified in terms of their ovarian activity immediately after the onset of puberty. Heifers selected on this basis required an average of 3,2 inseminations per calf born while 20% failed to conceive, as against only 1,5 inseminations per calf born for the rest and 95,5% conception.

Of great practical importance is the fact that the ovarian activity of individuals as heifers was to a remarkable extent reflected in terms of their fertility as adults. The importance of the elimination of heifers that are difficult to settle and the selection of heifers on the basis of functional efficiency, are clearly emphasized.

Ovulatory failure decreased fertility severely. Furthermore, at estrus periods with normal ovulations, more than twice the number of animals failed to conceive on a high feeding level than on a low feeding level. Out of 19 inseminations that failed with normal ovulations at estrus, 13 were in cows on a high level of feeding and only 6 were in cows on a low level of feeding.

Aberrations in cycle length were studied in relation to the absence or the presence of a corpus luteum during the same cycle. It was clear that where a corpus luteum was present, fertility appeared to be unaffected at the subsequent estrus period. With the absence of a corpus luteum on the other hand, fertility was significantly reduced in so far as an increased incidence in ovulatory failure was recorded.

Apart from the application of observations on cycle length in fertility work in the bovine, one is tempted to speculate on the possible endocrine mechanisms involved. It appears that a common factor might be involved in aberrations in cycle length and in ovulatory failure at estrus.

4.1.1 Estrus and palpation of the ovaries

Behaviour of animals at estrus was reported and practical problems encountered with the determination of the onset and the end of estrus were discussed.

The identification of follicles and corpora lutea on palpation was discussed in detail. The ripe follicle and the corpus luteum exhibit considerable variations on palpation, not only between animals but also between cycles in the same animal and even within the same cycle at different examinations. Variations in the shape, size and consistency of the same corpus luteum at different stages of its life-span were most apparent and the ease with which a soft and flabby corpus luteum could be confused, with a follicle on palpation, was obvious particularly with the presence of fluid in the corpus luteum at times.

The possible influence of the presence of fluid in the corpus luteum was recorded and considered in relation to reports on the efficiency of cystic corpora lutea. The concensus of opinion that the presence of fluid in a corpus luteum is incidental to its development and not deleterious to its function, was supported by observations in this investigation.

Cystic enlargement of the follicle and/or endometrium was encountered in 10% of animals in this study. Functional abberations preceded the development of cystic follicles and it appeared that these abnormalities were evident right from the onset of cyclic activity so that the condition is suspected to be congenital.

The high nutritional level employed in this investigation, had an overwhelming effect on follicular development and exerted a distinct gonodotrophic influence on ovarian activity which could be clearly illustrated on every day of the cycle and for each ovary. A sharp increase in follicular activity in the group on the high feeding level became evident from day 13 of the cycle, but in the group on the low feeding level, follicular activity increased only from day 18. Ovarian activity was further notably higher throughout the cycle in each of the two ovaries

in the high feeding group than in the low group, inclusive of the day of estrus and the period following immediately after estrus.

Since ovarian activity is stimulated by circulating gonadotrophin, the difference in response between the left and the right ovaries during the cycle and during successive cycles, remains a complete mystery. If the estrogenic function of follicles is considered in terms of a potential effect on ovum and sperm transport, a possibility is quite feasible of unripe or aged ova reaching the uterus, particularly since the average number of inseminations per conception and the conception rate were adversely affected by the higher nutritional level.

Further investigation on the estrogenic output of the ovaries in females on different nutritional levels and its effect on the fallopian tubes and uterus during fertilization and implantation, is indicated.

4.1.2 Temperature, heart and respiratory rates

Further to the stimulation of ovarian activity, the increased nutritional level was responsible for a notable acceleration in the heart and respiratory rates of the animals while body temperatures remained unaltered. Respiratory rates averaged 35 per minute in the low feeding group and 48 per minute in the high feeding group, with average heart rates 66 as against 81 per minute in the respective groups. On hot days average respiratory rates as a high as 60 per minute were recorded in the high feeding group as against 35 in the low feeding group while heart rates were 81 and 59 per minute respectively.

The effect of heart and respiratory acceleration *per se* could not be established in terms of the relative performance of the animals concerned. No differences have however been reported between environmental and digestive heat as stressors in the bovine. It can be argued therefore that digestive or fermentation heat most likely limits the performance of the bovine to the same extent as environmental heat, a subject that likewise merits further investigation since high nutritional levels are required in feedlots and in show animals and where breed differences are known to exist in the degree of heat tolerance.

4.1.3 Laminitis, arthritis and osteodystrophia

Laminitis, arthritis and osteodystrophia constituted one of the most severe forms of erosion in bovines on a high feeding level. Laminitis is well recorded in relation to the feeding level in the bovine. Breed susceptibility has been reported and the complete resistance of certain individuals within the breed (2 out of 12 in this study) has been conclusively demonstrated in this investigation in relation to laminitis, as well as arthiritis and other osseous changes that were recorded on the high feeding level.

No reports are available on arthritis or osteodystrophia as a herd problem in the bovine and it has never been reported in connection with a high feeding level. The aetiology of arthritis and osseous changes recorded in this study is unexplained. The occurrence of these conditions in association with laminitis might indicate a common cause. However, cases were recorded in animals that were not susceptible to laminitis.

Mechanical strain might be said to play a role but the symptoms in the bovine hardly reminds of complications in the horse where mechanical strain plays a prominent role.

4.1.4 Parturition and post parturient uterine regression

Dystocia presents a major problem in the highly fed Friesland female. In this study a 60% incidence of dystocia in cows on the high feeding level was recorded as against the complete absence of dystocia in the group on the restricted level of feeding.

Behaviour at calving differs notably between individual animals whether dystocia is evident or not. Behavioural reactions, relaxation of the cervix, the position of the fetus in relation to the pelvic outlet as well as the onset, frequency and intensity of physical labour all vary most considerably between individual animals at parturition whereby the determination of the exact stage at which assistance is required, is complicated.

The size of the fetus in relation to the pelvic opening is not the only factor of importance in dystocia in the over-fed bovine. Uterine inertia plays a role

and it prolongs the process of parturition notably, even where only mild assistance is required for delivery of the fetus, while one case of spontaneous rupturing of the uterus was recorded.

Prolonged labour prolongs the period required for uterine involution whereby a favourable environment is created for putrefaction of uterine contents and post parturient metritis. Average time of labour was 1 hour 24 mins. in the low feeding level group and 5 hours 20 mins. in the high feeding level group. Uterine regression took an average of 17 and 29,5 days respectively and the period to development of the first corpus luteum after calving was 20 and 85 days respectively for the low and the high feeding level groups. More than 50% of cows on the high level of feeding required intensive treatment for metritis after calving while not a single case of metritis was treated in the group on the low feeding level.

It appears therefore that myometrial exhaustion is a primary cause of post parturient metritis in which bacterial proliferation is an incidental complication.

4.2 Histological examination of the post partum uterus

4.2.1 Mobile cell infiltration

The first 14 days of the puerperium is characterized by a significant increase in numbers of neutrophiles, lymphocytes, eosinophiles, mast cells, plasma cells and histiocytes in the endometrium.

Neutrophile and histiocyte populations show notable similarities. A larger number of both cell types were recorded in the endometrium of the HIGH group than in the LOW group and with a more gradual return to normal levels. This indicates prolonged inflammatory changes in the endometrium of the HIGH group.

Lymphocyte numbers again resemble numbers of plasma cells in both groups. As with neutrophiles and histiocytes, the numbers of lymphocytes and plasma cells in the uteri of the HIGH group indicate a prolonged period of inflammatory changes.

Mast cell and eosinophile populations again showed notable similarities but here, cell numbers in cows on the lower feeding level exceeded those of the HIGH group with post partum complications.

Histological changes proceed after completion of palpable or clinical regression of the uterus. Palpable regression was complete after an average of 17 and 29,5 days in the LOW and HIGH groups respectively while histological changes proceeded until 28 or more days in the LOW group and 56 or more days in the HIGH group.

The exact end point of involution is somewhat vague. The return to normal cell numbers in the endometrium was complete at different stages after calving for different cell types in each of the two groups studied.

It is clear that cows on a high feeding level are subjected to a more prolonged period of uterine regression after calving. Delayed regression follows not necessarily on dystocia but also on an increased period of labour in which uterine inertia plays a role.

Prolongation of uterine regression can be recognized not only in relation to the palpable size of the uterus, but also in mobile cell infiltration which proceeds after completion of palpable regression.

The grouping together of mobile cell types is a regular feature of the post partum endometrium. This can be observed as dense packing of certain capillaries with a specific cell type or perivascular concentration of these cells while adjacent capillaries might be free from such cells. This indicates that concentrations are quite localized.

It has been observed that mast cells sometimes concentrate around lymph follicles that develop in the endometrium. Sometimes a single row of mast cells is observed to encircle a lymph follicle or to occur immediately below the surface epithelium.

Occasionally a ten- to twenty-fold increase in numbers of certain of the more rare cell types (mast cells, plasma cells or eosinophiles) has been observed with a return to normal numbers in 7 to 14 days. These increases could not be related to any clinical event.

4.2.2 Surface epithelium

Attachment of the surface epithelium is loose and it is often deranged in a biopsy specimen. The surface epithelium is frequently invaded by migratory

neutrophiles, lymphocytes or histiocytes and the numbers of these migratory cells follow the general pattern of the presence of these cells in the rest of the endometrium.

Plasma cells, mast cells and eosinophiles were not observed to migrate through the surface epithelium.

Vesicularity of the nucleus was also associated with an increase in cell height and was at a maximum 8 to 14 days after calving. Cows on the higher level of feeding exhibited more vesicularity of the nucleus and increased cell height during this period as well as during estrus, than cows on the lower feeding level. It is possible that the increase in follicular activity on the high feeding level, might have induced this effect on the surface epithelium.

The higher level of vesicularity of the nucleus and the higher level of ovarian activity during the estrus cycle in cows on the ad libitum level of feeding might be related to the lowered reproductive efficiency of this group, particularly, where normal ovulatory performance was shown to result in failure of fertilization after insemination in the HIGH group to a much greater extent than in the LOW group.

It is surmised that an incorrect stage of maturation of the ovum at fertilization or an unfavourable uterine environment was induced by hormonal imbalances or excesses created by excessive feeding.

An individual rhythm in cell height and vesicularity of the nucleus of the surface epithelium of the endometrium was most apparent. Large vesicular nuclei were frequently seen adjacent to dark and elongated pyknotic nuclei in the same microscopical field so that variations within specimens frequently over shadowed variations between specimens.

4.2.3 Endometrial glands

Endometrial glands exhibit notable variations in cell height and vesicularity of nuclei, lumen size and debris or cellular content of the lumen. Variations within sections overshadow variations between sections so that an individual rhythm is obvious as with the surface epithelium. Migration of mobile cells between the glandular epithelium and into the lumina of the glands follows the pattern of

migratory cells in the rest of the endometrium and no differences in endometrial glands are apparent between cows on a high and a low level of feeding or between estrus and the luteal phase of the cycle.

Glandular sections close to the endometrial surface regularly show greater cell height and more vesicularity of the nucleus than sections deeper in the endometrium where nuclei were generally dark and pyknotic with lower cells. Glands towards the surface therefore show greater activity than glands deeper in the stroma of the endometrium.

4.2.4 Lymph follicles in the endometrium

Lymph follicles frequently develop in the endometrium about 12 to 15 days after calving to disappear again after 2 or 3 weeks. Follicles are unrelated to any clinical event and were recorded in completely normal endometria just as frequently as in cases of prolonged uterine regression or metritis.

The presence of lymph follicles in the endometrium was always associated with relatively high counts of lymphocytes and plasma cells throughout the endometrium.

Summarily it must be recorded that in the histological examination of the endometrium, the demarcation between pathological and physiological states of the endometrium is delicate. Increased numbers of cells, particularly of a specific type, are apparently often extreme variates in a normal physiological variation.

4.3 Body composition and clinical tests

Prolonged over-feeding does not induce any obvious long term excesses or deficiencies or any clinical pathological abnormalities over extended periods, but modification of certain metabolic pathways are suspected and particularly the acceleration of certain processes.

Marked individual differences between cows are most apparent in relation to all the observations and all the estimations that were made. These differences were frequently independent of the level of nutrition.

Between the two feeding levels that were employed, differences were even more dramatic. A variety of most significant conditions developed in the group

on the high level of feeding, and this constitutes the very crux of this investigation.

Laminitis and arthritis with osteodystrophia, uterine inertia, dystocia, spontaneous rupturing of the uterus as well as a notable delay in uterine regression with a severe incidence of post partum metritis were rife. Fatal metabolic disease as well as a frequent incidence of subclinical cases of localized pleurisy and peritonitis, interstitial nephritis, fatty necrosis and proliferative endocarditis were further recorded on the high feeding level.

Numerous reports are available on these conditions; their aetiology and control measures are well described. However, a common linkage between these conditions has never been suggested.

This investigation has proved the existence of such a linkage. It has shown that marked differences exists between animals of the same type and on the same feeding level, and that a *locus minoris resistenstiae* operates for each individual which, when the metabolic processes are accelerated by an increase in feeding level, breaks down and terminates the productive life-span of the animal prematurely.

It has also been shown conclusively that the bovine is not able to adjust its nutritional intake according to its physiological requirements. High feed intake is essential in certain aspects of animal production but some breeders do not hesitate to employ *ad libitum* levels of feeding for breeding purposes and in particular in the show conditioning of animals, amongst which highly esteemed breeding stock naturally resorts.

This investigation shows conclusively that such standards are tantamount to dire malpractice. It is of great importance therefore that, at all levels of feeding, the upper as well as the lower limits of an animals requirements be clearly laid down, particularly as it was so clear in this study that the upper feeding level was completely unjustified in terms of milk production.

4.4 Conclusions

* The incidence of ovulatory failure in the form of anovulation and delayed ovulation in dairy heifers, from the onset of cyclic activity until conception, can occur at 43% of estrus periods and 23% of estrus periods after the first calving.

* The proportion of dairy heifers that exhibits a normal ovulatory pattern at each estrus period from the onset of cyclic activity until conception might be as low as 8% while the proportion of heifers exhibiting normal ovulations at more than one half of the total number of corresponding estrus periods, might be as low as 56%.

* The incidence of ovulatory failure in heifers, is reflected in their fertility as adults and consequently it is of importance to eliminate heifers that are difficult to settle.

* Ovarian activity and follicular development in the ovulating as well as the non-ovulating ovaries are increased on every day of the estrus cycle by an increased level of feeding.

* Reduction of feed intake in heifers on *ad libitum* feeding 6 weeks before breeding, results in a reduction in the number of services per conception.

* Cows on *ad libitum* feeding require a larger number of inseminations per conception than cows on a controlled level of feeding.

* In cows on *ad libitum* feeding a larger proportion of cows fail to conceive on insemination at estrus periods where ovulation takes place within the normal time from the onset of estrus, than in cows on controlled feeding. This suggests that estrogenic levels from increased follicular activity from high feeding levels might affect ovum transport.

* Over-feeding in the bovine, might be responsible for a serious incidence of dystocia at calving in which primary uterine inertia plays a prominent role.

* The duration of labour and the time required for completion of uterine regression and for the development of the first corpus luteum after calving respectively, are significantly prolonged in overfed cows while the incidence of post parturient metritis is greatly increased.

* The first 14 days of the puerperium in the bovine is characterized by a significant increase in the respective numbers of neutrophiles, mast cells, lymphocytes, eosinophiles and histiocytes in the endometrium. The numbers of all these cell types are significantly higher in over-fed cows than in cows on controlled levels of feeding.