The effect of dietary protein on reproduction in the mare. III. Ovarian and uterine changes during the anovulatory, transitional and ovulatory periods in the non-pregnant mare

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ABSTRACT

In the main experiment the total daily protein intake and quality (essential amino-acids) was varied in 4 groups of mares. The incidence of oestrus in mares during the transitional period was unaffected by protein nutrition. Ovarian activity, as evaluated by follicular development and size of the ovaries, was affected. Mares that received low-quality protein (Groups 1 and 2) had a higher number of smaller follicles (<10 mm) that developed during the transitional period compared to mares on a high-quality protein intake (Groups 3 and 4). The mares that received the high quality protein ovulated 2–3 weeks earlier in the breeding season in a synchronised period of 4–5 weeks compared to a period of 6–8 weeks in Groups 1 and 2. The duration of the subsequent oestrous cycles was not affected. There was no difference in the diameter of the largest follicle of mares between groups on the day before ovulation. In a separate experiment, 5 maiden Anglo-Arab mares, 4–5 years of age, were slaughtered at different stages during the anovulatory, transitional and ovulatory periods of the breeding cycle. The morphology of the ovaries and uteri of these mares was described and photographed for use as guidelines when comparing ovarian changes and follicular activity of mares.

Key words: equine, oestrus, ovulation, protein nutrition, transitional period.

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INTRODUCTION

Foals are born throughout the year, which implies that the mare is polyoestrous with a tendency to the non-oestrous state[9]. Investigations in both the northern and southern hemispheres on the incidence of oestrus, ovarian activity and ovulation in mares of different breeds indicate that 80–100 % of mares ovulate during the mid-summer months but the percentage decreases to 20–25 % during the winter months[4–7,9]. According to these findings, mares can be classified into 3 groups. The 1st group comprises mares that remain cyclic throughout the year. The 2nd group comprises mares with a definite anoestrous period with small inactive non-cyclic ovaries during the winter months, and a definite ovulatory period during summer. The last group comprises mares that do not return to a completely anoestrous state. The ovaries of these mares remain active throughout winter, with waves of follicles that develop but undergo atresia without ovulation[1,9].

During the period May to August the mean duration of oestrus is reported to be 20 days with a range of 5–38 days[10]. During spring (August–October) the average duration of oestrus is 13.7 d and in summer (November–February) 5.2 d[7,8,10].

The major external factors influencing the sexual activity of the broodmare are probably photoperiod and nutrition. Mares further from the equator have a more pronounced winter anoestrous period[2,7].

Although photoperiod plays an important role in the sexual activity of the mare, it was not possible to stimulate ovarian activity by increasing the photoperiod in mares suffering from under- or malnutrition[5,11]. Critical documentation of the effect of nutrition on ovarian activity in mares is limited. Energy supplementation increased ovarian activity in a 3-week study[3], while in another study an increased ovulation rate was found when the diet of mares was supplemented with high-quality protein early in the breeding season[11].

The anovulatory period is described as the time from the last ovulation during the ovulatory period of the breeding season until the 1st ovulation of the following ovulatory (breeding) period. During this time the mare may come into oestrus, particularly during the transitional period that precedes the ovulatory period. The mare will allow mating, but does not ovulate. During this anovulatory period, which includes the period of ovarian transition, rectal palpation and ultrasonography to monitor the size of the ovaries and the size and consistency of the follicles are the preferred methods to determine the degree of ovarian activity.

The purpose of this study was to determine the effect of protein nutrition on follicular development during the transitional period until the 1st ovulation.

MATERIALS AND METHODS

Experiment 1

Five Anglo-Arab mares, aged 4–5 years, were slaughtered during the anovulatory, transitional and ovulatory periods. These mares were teased and their ovaries and uteri palpated daily per rectum for a period of at least 1 month before the 1st mare was slaughtered. The estimated size and consistency of the ovaries and follicles palpated rectally were recorded immediately before slaughter. Immediately after slaughter the reproductive tract was removed and the visual appearance, consistency and actual size of the ovaries, follicles and uteri recorded and photographed. The walls of selected follicles were dissected and processed for histological examination as described by Van Niekerk[12].

Experiment 2

In the 2nd experiment 36 Anglo-Arab and Thoroughbred, non-lactating mares were divided according to age and body mass into 4 nutritional groups of 9 mares each where the feed varied in the essential amino-acids and total protein content. The composition and analysis of the rations were as follows:

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Group 1: tef hay (5 kg), cubes (2 kg), (crude protein 10.8 %, threonine 0.30 %, methionine 0.11 %, iso-leucine 0.38 %, leucine 0.71 %, lysine 0.55 %, arginine 0.62 %).

Group 2: lucerne hay (5 kg), cubes (2 kg), (crude protein 14.0 %, threonine 0.5 %, methionine 0.10 %, iso-leucine 0.62 %, leucine 1.04 %, lysine 0.76 %, arginine 0.62 %).

Group 3: tef hay (5 kg), cubes (2 kg), fishmeal (0.2 kg), (crude protein 12.06 %, threonine 0.35 %, methionine 0.15 %, iso-leucine 0.43 %, leucine 0.68 %, lysine 0.65 %, arginine 0.59 %).

Group 4: lucerne hay (5 kg), cubes (2 kg), fishmeal (0.2 kg), (crude protein 15.15 %, threonine 0.53 %, methionine 0.14 %, iso-leucine 0.66 %, Leucine 1.00 %, lysine 0.86 %, arginine 0.94 %).

The management, feeding procedures and teasing programme of the mares, the analytical methods used in the chemical analyses of the rations, and the daily crude protein and amino-acid intake of the mares in the different groups remained the same as described previously in the second article in this series[13].

From 1 July to 31 March the following year all mares were teased daily. During the period 1 July to 20 August the ovaries of each of the 36 non-lactating mares were palpated rectally weekly. Ovaries of mares that came into oestrus after 21 August were palpated rectally on that day and daily thereafter while the mare showed signs of oestrus. The ovaries of mares that showed no signs of oestrus continued to be palpated weekly to determine follicular activity. The estimated size of the ovaries and palpable follicles was recorded at each examination. The number of follicles larger than 10 mm in diameter was recorded. Rectal findings of follicular size were confirmed by ultrasonography. Approximately 95 % of all examinations were performed by the senior author.

Mares were mated under controlled conditions when a 30–50 mm soft, thin-walled follicle was present. Mares were mated and examined rectally daily thereafter until ovulation occurred.

RESULTS

Only the *post mortem* results of the morphology of the ovaries and uteri of the 5 mares slaughtered at different stages of the breeding cycle are given, as these results correlated very closely with the findings of the rectal palpations performed immediately before the mares were slaughtered.

Mare A was slaughtered in the 1st week of June (anovulatory period). This mare was in deep anoestrus and had not shown any signs of oestrus for a minimum of 2 months before slaughter. *Post mortem* findings (Figs 1A, 2A): size of left ovary was $21 \times 15 \times 15$ mm and right ovary $23 \times 15 \times 15$ mm. Both ovaries were kidney-shaped and firm, with a smooth surface with no sign of follicular activity. The walls of the uterine horns were very thin and without tonus. The ovaries of this mare were classified as completely inactive, smooth, non-cyclic (ISN) and ovaries of this type are usually found in mares in deep anoestrus (Table 4).

Mare B was slaughtered in the 3rd week of July. No signs of oestrus were recorded for a period of 2 months before slaughter. The left ovary measured $33 \times 25 \times 21$ mm (Fig. 1B) and the right $31 \times 23 \times 21$ mm. Both ovaries were relatively small but larger than those of Mare A and kidney-shaped. The consistency was firm and the surfaces slightly uneven but not knobbly. There were no measurable follicles protruding above the ovarian surfaces. These ovaries were classified as inactive, smooth, non-cyclic (ISN). The walls of the uterine horns were relatively thin without tonus when palpated rectally before slaughter.

Mare C (Fig. 1C) was slaughtered in the 2nd week of August (beginning of the transitional period). No signs of oestrus were recorded for at least 2 months before slaughter. The left ovary measured $43 \times 34 \times 30$ mm and the right ovary $42 \times 35 \times 30$ mm, both much larger than the ovaries of Mares A and B. The ovaries were more or less round in shape with knobbly surfaces. Several follicles ($n = 13$) <10 mm in diameter protruded above the ovarian surfaces. These ovaries were classified as active, non-cyclic ovaries (AKN). Histological examination of the walls of the largest follicles (Fig. $1D_2$) revealed that the *theca interna* layer was not as clearly developed as that of a Graafian follicle (Fig. $1E_1$). The granulosa cells (Fig. $1D_2$) had small inactive pycnotic nuclei that were densely packed in a thin layer, indicating that these cells were still inactive and that probably very little oestrogen was secreted in the follicular wall[12]. The uterine wall was relatively thin and without tone.

Mare D (Figs 1D, D_1, D_2; 2D) was slaughtered in the 1st week of September, late in the transitional period. This mare had been in oestrus for a period of 28 days. Rectal palpations and serum progesterone levels had been performed daily, which showed that this mare had not ovulated during this 28-day period. The left ovary measured $68 \times 51 \times 50$ mm and the right ovary $65 \times 52 \times 50$ mm. Both ovaries were large and knobbly, containing several protruding follicles <25 mm in diameter (Fig. 1D). The largest follicles had relatively thin walls and fluctuated on palpation. The visual and histological examination of the internal wall of these largest follicles revealed that these follicles underwent atresia (Fig. $1D_1$, D_2). The inner wall of the atretic follicles appeared pale without the presence of blood vessels in contrast to the inner wall of an active, developing Graafian follicle as described in Mare E. In the large atretic follicles the granulosa cells as well as the cells in the *theca interna* layer undergo degeneration and are sloughed off into the antrum of the follicle, where they pack together to form round dark pycnotic bodies called atretic bodies. It is obvious that during the transitional period several follicles developed up to the early Graafian stage, the so-called LH-dependent stage, and then underwent atresia because insufficient LH was secreted at this stage to induce ovulation. This will be supported and explained in a later publication in this series. The ovaries of this mare were classified as active, non-cyclic, with large follicles (AGKN) (Table 4).

Mare E (Figs 1E, 2E) was slaughtered in the 1st week in November, 18 days after ovulation, approximately 2 days before the next ovulation. She had shown regular oestrous cycles of 20 days' duration.

Post mortem findings (Figs 1E, 2E): the left ovary measured $74 \times 54 \times 42$ mm. A large, thin-walled follicle of 42 mm in diameter was present. The right ovary measured $45 \times 35 \times 27$ mm in diameter. A degenerating corpus luteum of the previous ovulation of 15×16 mm was present as well as a few small follicles <10 mm in diameter. A dense network of small blood vessels was noticable on the inner wall of the large Graafian follicle, in contrast to the lack of blood vessels on the pale bloodless inner wall of large atretic follicles (Fig. $1D_1$).

Histologically the wall of the Graafian follicle consisted of a granulosa layer (g), basement membrane (bm), *theca interna* (ti) and *theca externa* (te) (Fig. $1E_1$). The nuclei of the granulosa cells were large and vesicular. The nuclei of the *theca interna* cells were large, vesicular and contained well-developed nucleoli. The cytoplasm of these cells had a granular appearance, which is a sign that these cells were actively secreting oestrogens. The ovaries of this mare were classified as active, cyclic. On palpation, the uterine horns were enlarged compared with those of Mares C and D, soft and flabby, and were not contracted.

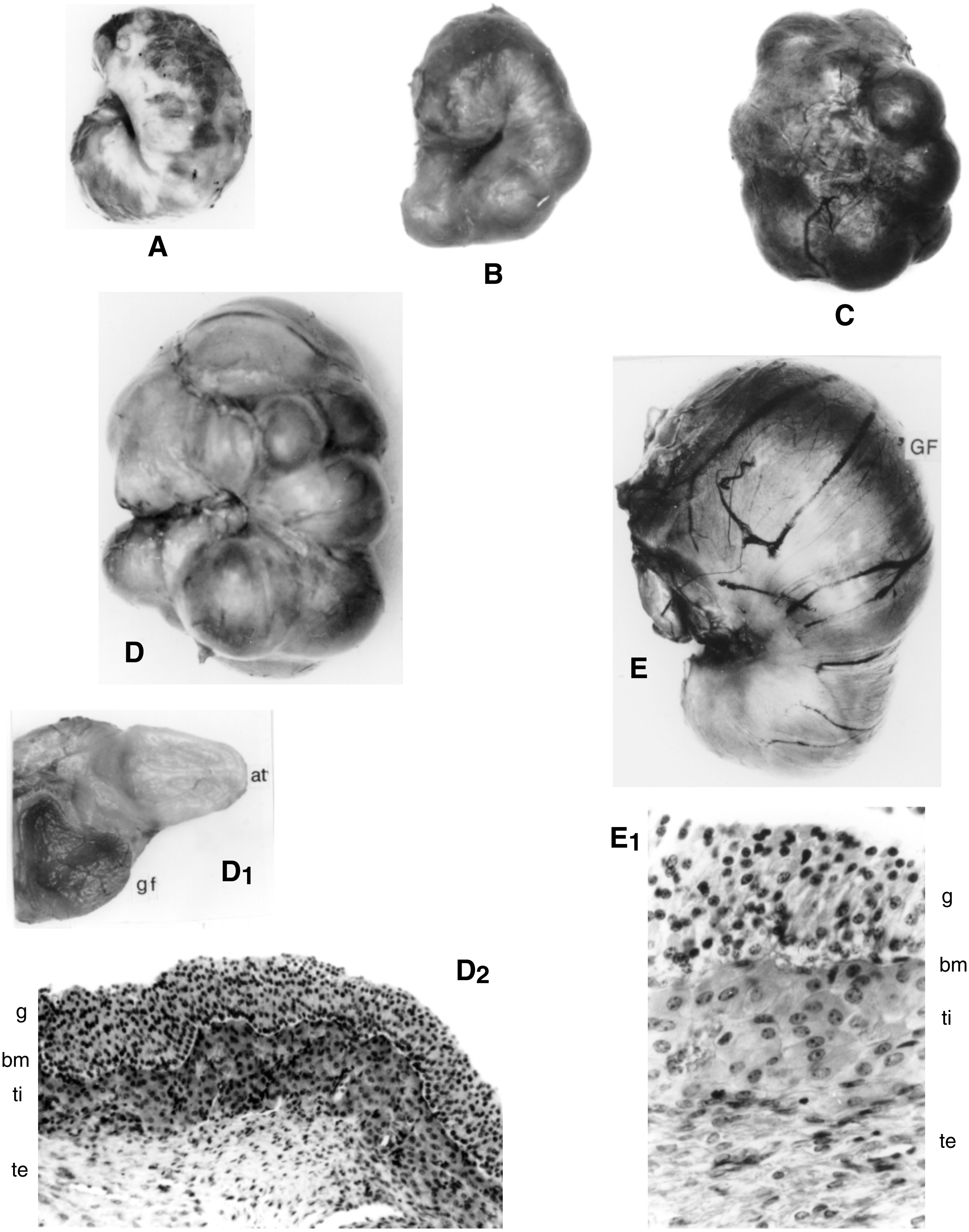

Fig. 1:

A: **inactive, smooth, non-cyclic ovary** (ISN) **of Mare A in deep anoestrus;**

B: **inactive, smooth, non-cyclic ovary** (ISN) (surface slightly uneven but not knobbly) **of Mare B in anoestrus;**

C: **active, non-cyclic ovary** (AKN) **of Mare C in anoestrus in the transitional period. Note large number of follicles <10 mm in diameter;**

D: **active, non-cyclic ovary of Mare D that showed an abnormally long oestrous period during the late transitional period. Note the large number of developing and atretic follicles <25 mm in diameter** (AGKN);

D_1: **section through the ovary of Mare D showing the inner walls of an atretic** (at) **and a large developing follicle** (gf);

D_2: **a histological section of the wall of a large developing follicle of Mare D** (HE ×80). (g = granulosa layer; bm = basal membrane; ti = *theca interna*; te = *theca externa*);

E: **an active, cyclic ovary of Mare E showing a large Graafian follicle** (GF) **approximately 1–2 days before ovulation**;

E_1: **histological section of the wall of the large Graafian follicle of Mare E** (HE ×400). (g = granulosa layer; bm = basal membrane; ti = *theca interna*; te = *theca externa*).

In the main experiment the mean percentage of mares that showed signs of oestrus daily from 1 July to 28 September was 18, 44, 30 and 35 % for Groups 1, 2, 3 and 4 respectively. No ovulations occurred during this period. Some mares showed signs of oestrus for several weeks, while other mares only showed signs of oestrus for a few days. The mean duration of the oestrous periods associated with the 1st ovulation after the anovulatory period and the mean duration of the following oestrous periods are given in Table 1. The time and cumulative percentage of mares that ovulated for the first time after the anovulatory period are given in Table 2. The duration of the oestrous cycles following the 1st ovulation of mares in the different groups is given in Table 3. Several mares were served during this 1st ovulatory oestrous period following the anovulatory period.

Table 1: **Comparison of the mean duration of the oestrous periods associated with the 1st and subsequent ovulations**.

Group	Mean duration of the oestrous period (days)	
	First ovulation	Later ovulations
1	17.3 ± 9.6 (8–33) *n* = 6[a]	6.0 (6.0) *n* = 2
2	15.9 ± 8.2 (7–33) *n* = 8	7.6 ± 4.2 (4–16) *n* = 7
3	19.6 ± 5.1 9–25 *n* = 8	8.6 ± 3.4 (3–17) *n* = 12
4	15.5 ± 8.1 (7–28) *n* = 8	7.1 ± 2.6 (2–12) *n* = 10
Mean	17 days (*n* = 30)	7.3 days (*n* = 31)

[a]*n* = number of ovulations.

DISCUSSION

There were no differences in the duration of the oestrous periods between groups; the results were similar to the average duration of 13.7 d during autumn and the 7.3 d in summer as reported in the literature[7,8,10].

Two important observations emanated from these results. Mares in Group 1 (tef hay and cubes) commenced ovulating 2–4 weeks later in the breeding season than the 3 groups that received higher quality protein in their diets. Secondly, a cumulative ovulation percentage of 90 % was reached within 5 weeks after the 1st ovulation in Groups 3 and 4, which received the fishmeal supplementation, in contrast to the mares in Groups 1 and 2 that only reached the same cumulative ovulation rate 2–3 weeks later. Comparison of the results of the different groups shows clearly that a high-quality protein diet not only had a synchronising effect on the timing of the 1st ovulations in the breeding season, but also resulted in ovulation occurring approximately 3 weeks earlier. These results indicate that the quality of protein in the diet directly influences the mechanisms involved in the onset of the ovulatory season, which is of considerable practical importance, since in effect it adds an extra oestrous cycle in the limited breeding season.

No differences in the duration of the oestrous cycle during the ovulatory period were found between groups, and the variation in cycle duration (17–33 d) is in agreement with the literature[7,9].

Table 2: **Cumulative percentage of mares in the 4 dietary groups that ovulated for the first time after the anovulatory period**.

Week of the month		Cumulative percentage of mares that ovulated			
		Group			
		1	2	3	4
Aug	4	0	11	0	0
Sept	1	0	33	0	0
	2	0	44	12	11
	3	0	44	12	11
	4	28	44	38	22
Oct	1	28	55	75	44
	2	28	55	88	89
	3	43	66	88	89
	4	57	66	100	89
Nov	1	86	89	100	100
	2	86	100	100	100

Table 3: **Mean duration of oestrous cycles (days) in mares in each of the 4 groups from September to December**.

Group	Duration of oestrous cycle (days)
1	24 ± 7.4 (17–33) *n* = 4[a]
2	22 ± 2.5 (18–25) *n* = 7
3	23 ± 3.3 (19–31) *n* = 12
4	22 ± 3.2 (19–31) *n* = 12

[a]*n* = number of oestrous cycles.

Ovarian changes

The results presented in Table 2 show clearly that the largest difference in the

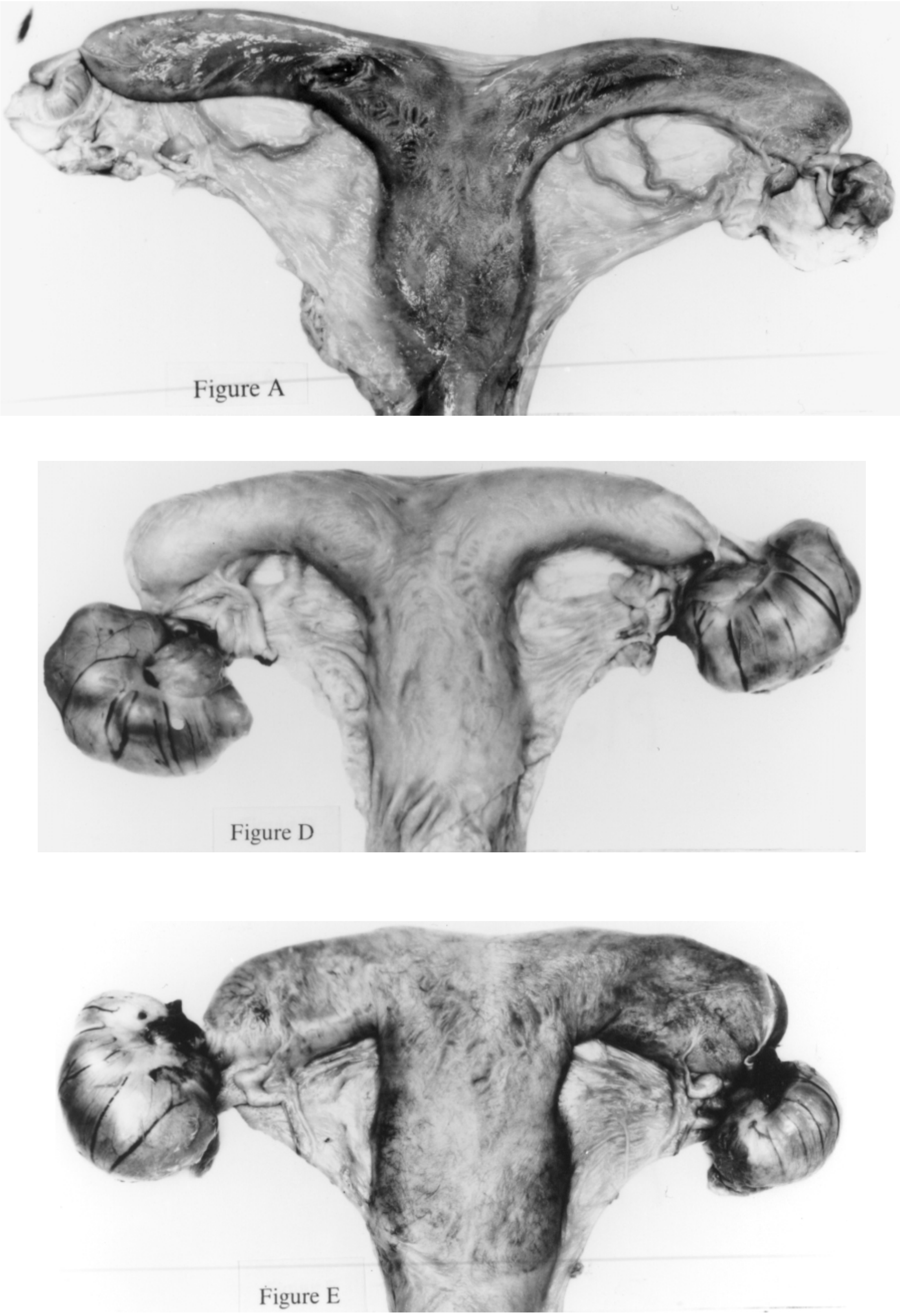

Fig. 2:

A: **uterus and ovaries of a mare in deep anoestrus during the anovulatory period July. Both ovaries are small, kidney-shaped and hard. The ovarian surface is smooth without any signs of follicular development. These ovaries are classified as inactive, smooth with no palpable follicles** (ISN). **The body and horns of the uterus are thin and without tonus**;

D: **uterus and ovaries of a mare, in the transitional anovulatory phase** (August–September). **Both ovaries are large, rounded and knobbly with several follicles of 10–25 mm in diameter. The walls of the body and horns of the uterus are thickened and have reasonable tone**;

E: **uterus and ovaries of a mare (Mare E) in oestrus 1–2 days before ovulation during a normal oestrous cycle. A large Graafian follicle** (42 mm) **is present in the left ovary. The remains of a *corpus luteum* are present in the right ovary. The uterine wall is thickened but has less tone than that of Mare D, in oestrus for 28 days without ovulation.**

Table 4: **Ovarian activity in mares that received no fishmeal supplementation** (Groups 1 and 2) **and those that received fishmeal** (Groups 3 and 4) **during July and August**.

Date	Ovarian activity (%) of mares					
	ISN		AKN		AGKN	
	NF	F	NF	F	NF	F
3/7	72.2	77.8	22.2	8.3	5.6	13.9
10/7	57.9	69.5	28.3	22.2	13.8	8.3
17/7	58.4	63.9	22.2	19.4	19.4	16.7
24/7	55.7	36.4	27.7	46.9	16.6	16.7
31/7	44.5	53.1	33.3	30.2	22.2	16.7
7/8	58.4	50.0	25.5	41.6	11.1	8.4
14/8	66.7	66.7	22.2	33.3	11.1	0
21/8	61.4	74.9	16.3	25.1	22.3	0
28/8	50.0	66.5	38.6	24.5	11.3	0

NF = no fishmeal (Groups 1 and 2) (n = 18).
F = fishmeal (Groups 3 and 4) (n = 18).
ISN = inactive, smooth non-cyclic ovaries with no palpable follicles (Fig. 1B).
AKN = active, knobbly non-cyclic ovaries with follicles not larger than 10 mm (Fig. 1C).
AGKN = active, non-cyclic ovaries with follicles not larger than 25 mm (Fig. 1D).

Table 5: **Graafian follicle diameter** (mm) **on the day before the first and subsequent ovulations of the mares in the 4 groups**.

Group	Follicle diameter (mm) on the day before ovulation	
	First ovulation	Later ovulations
1	41 ± 8.2 (30–55) n = 6	37 ± 10.6 (30–45) n = 2
2	43 ± 9.6 (30–55) n = 9	44 ± 5.3 (40–55) n = 7
3	36 ± 5.2 (30–45) n = 8	36 ± 8.8 (30–55) n = 12
4	41 ± 9.2 (25–55) n = 9	39 ± 7.0 (30–45) n = 10

cumulative percentage of mares that ovulated after the anovulatory period was found between Groups 1 and 2 (no fishmeal), and the fishmeal-supplemented Groups 3 and 4. These results indicate that, throughout July and August, both the fishmeal- and non-fishmeal-supplemented groups exhibited a similar percentage of inactive, smooth, non-cyclic ovaries (ISN) with no follicles (Fig. 1B). It is clear that during the transitional period from the beginning of August considerably more follicular activity was found in the fishmeal-supplemented Groups 3 and 4 than in the non-fishmeal-supplemented Groups 1 and 2. On 7 August 41.6 % mares in the fishmeal-supplemented groups (Groups 3 and 4) had active ovaries (Fig. 1C), compared with 25.5 % of mares in the non-fishmeal-supplemented Groups 1 and 2. From 7–28 August there was a steady decrease from 41.6 % to 24.5 % in the percentage of active non-cyclic (AKN) ovaries (Table 4) in the fishmeal-supplemented groups but not in the non-fishmeal-supplemented groups. This indicated that the ovaries of the mares in the non-fishmeal-supplemented groups generally remained active but non-cyclic, while the ovaries of the fishmeal-supplemented group became progressively more active during this transitional stage to the ovulatory stage when Graafian follicles developed (Fig. 1E). Consequently, these mares also ovulated much earlier in the breeding season than the mares that did not receive fishmeal. At the beginning of August only 8.4 % of mares in the fishmeal-supplemented groups were found to have large, active, knobbly, non-cyclic ovaries (AGKN), and by 14 August (late transitional period) none of these mares demonstrated this ovarian classification. In direct contrast, the ovaries of 11.1 % of the mares in the non-fishmeal-supplemented groups remained in a non-cyclic (AGKN) transitional state until the end of August (Table 4). The effect of fishmeal supplementation becomes even more apparent when the occurrence of active but non-cyclic ovaries with large atretic follicles up to 25 mm in diameter (AGKN) (Fig. 1D; Table 4) are studied.

It is clear from these results that the ovaries of mares that received a high-quality protein, rich in essential amino-acids, in their diet, moved rapidly from the inactive stage through the transitional stage to the ovulatory period and did not form large numbers of developing and atretic follicles, a characteristic of the non-fishmeal-supplemented group (Fig. 1D). It is also obvious that the mares that received fishmeal ovulated in a more synchronised manner and earlier in the breeding season, and that the source of the roughage, tef hay or lucerne hay played an insignificant role.

The minimal differences that were found in the mean diameter of the Graafian follicles on the day before ovulation in the mares in the 4 dietary groups (Table 5) indicate that the nutritional status of the mares had no effect on Graafian follicle size.

CONCLUSION

The inclusion of high-quality protein (fishmeal) in the diet of barren mares had a distinct effect on sexual behaviour and ovarian activity during the transitional period. Ovaries of mares on a high quality protein diet became more active earlier in the transitional period than those of mares fed a diet with lower quality protein. The ovaries of these mares also transformed quickly through the late transitional to the ovulatory stage and did not develop large numbers of growing and atretic follicles that characterised the ovaries of the groups not fed fishmeal. It is suggested that the high quality protein stimulates the secretion of LH at a much earlier stage in the breeding season and stimulates follicular development to the Graafian follicle stage and ovulation. Mares on the high quality protein diet also ovulated 2–3 weeks (or a full cycle) earlier in the season, and approximately 90 % of these mares ovulated within a short period of 4–5 weeks. In the non-fishmeal-supplemented groups the follicles only developed to the LH-dependent stage and, because insufficient LH was secreted, they did not develop to the ovulatory stage but underwent atresia.

Thoroughbred breeders generally are anxious that their mares should conceive as soon as possible after 1 September, and these results clearly indicate that supplementation of the diet with fishmeal (high-quality protein) from the beginning of the transitional period in July has a marked effect on the onset of the ovulatory breeding period, and consequently results in increased conception rates early in the official breeding season.

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REFERENCES

1. Allen W E 1979 Abnormalities in the oestrous cycle in the mare. *Veterinary Record* 104: 166–167
2. Arthur G H, Allen W E 1972 Clinical observations on reproduction in a pony stud. *Equine Veterinary Journal* 5: 109–117
3. Bengtsson G, Knudsen O 1963 Feed and ovarian activity of trotting mares in training. *Cornell Veterinarian* 53: 404–411
4. Doms H W H 1972 The oestrous cycle of the mare and some related phenomena. MSc thesis, University of Stellenbosch
5. Ginther O J 1974 Occurrence of anestrus, estrus, diestrus and ovulation in mares over a 12 month period. *American Journal of Veterinary Research* 35: 1173–1179
6. Osborne V E 1966 An analysis of the pattern of ovulation in the annual reproductive cycle of the mare in Australia. *Australian Veterinary Journal* 42: 149–154
7. Osborne V E 1975 Factors influencing foaling percentages in Australian Mares. *Journal of Reproduction and Fertility* Supplement No. 23: 477–483
8. Marlow C H B 1983 The oestrous cycle, mating practices, conception rates and fetal losses in Thoroughbreds in the Eastern Cape Province. MSc thesis, University of Stellenbosch
9. Van Niekerk C H 1965 The breeding cycle, ovarian changes and tubal sojourn of ova in the mare. M Med Vet thesis, University of Pretoria
10. Van Niekerk C H 1967 Pattern of the oestrous cycle of mares II. The duration of the oestrous cycle and oestrous period. *Journal of the South African Veterinary Medical Association* 38: 299–307
11. Van Niekerk C H, Van Heerden S J 1972 Nutrition and ovarian activity of mares early in the breeding season. *Journal of the South African Veterinary Association* 43: 355–360
12. Van Niekerk C H 1973 Morphology and physiological changes in the genital system of mares. DVSc thesis, University of Pretoria
13. Van Niekerk F E, Van Niekerk C H 1997 The effect of dietary protein on reproduction in the mare. II. Growth of foals, body mass of mares and serum protein concentrations of mares during the anovulatory, transitional and pregnant periods. *Journal of the South African Veterinary Association* 68(3): 81–85