CHAPTER 3

3.1 Material and methods

3.1.1 Experimental design and analyses

Eight Holstein-Friesian bulls aged between 2 ½ and 3 years were required from the experimental farm at the University of Pretoria, South Africa. They were all half brothers from the same sire and were of similar weights. The bulls were randomly divided into two groups of four. The control group was fed a diet of concentrates and *Eragrostis tef* hay as roughage with no vitamin E supplementation. The treatment group was fed the same diet as the control group but the diet was supplemented with Rovimix E provided by Roche Animal Nutrition and Health. The vitamin E supplementation was calculated according to previous research results (Kozicki *et al,* 1981 and McDowell *et al*, 1996) and the suppliers recommendations. The recommendation was that the bulls receive 1000 IU per day but due to the bioavailablity of the supplement being 50% the effective vitamin E supplementation was 500 IU per day per animal which is in accordance with the literature (McDowell *et al*, 1996). Both groups had access to fresh, clean water *ad libitum*.

All the animals were fed the control diet for approximately six months before the treatment commenced to ensure that the bulls were properly adapted to the diet. Thereafter four of the bulls were treated for 90 days with 100IU of vitamin E. Semen was collected every second week via artificial vagina. The artificial vagina was used because it is quick and a method available for

collection of semen (Bearden and Fuquay, 1997). Collections were done every second week to allow for a week of rest between collections to ensure that libido remains high and that the quality of the semen samples are not affected by exhaustion. The treatment commenced in October 1998 and was ended in December 1998 which falls in to the normal breeding season. The average temperature over the three experimental months reached a maximum of 23.3°C and an average minimum of 13°C with an average rainfall of 100.7mm (Pretoria Weather Bureaux). A complete semen evaluation of each bull was conducted after each collection, including quantification of volume, colour, motility, concentration, contaminants and morphology. Eosin and nigrosin was used for staining dead and live sperm. Eosin is referred to as a differential stain in that it cannot pass through living cell membranes but it can pass through non - living cell membranes. A background stain such as nigrosin helps make the unstained sperm heads visible.

The semen characteristics were divided in to three categories, namely the percentage normal spermatozoa (Figure 3.1), the percentage major semen defects, and percentage minor semen defect.

Major defects are those that relate to impaired fertility or to an abnormal condition in the epididymis (Blom as cited by Salisbury *et al* 1978) and include:

Teratoid sperm (TERAT) which is where the midpiece lies over the sperm head in a bent or partially coiled form (Figure 3.2),

- Knobbed acrosomes (AKR) which is characterised by a localised swelling or bead on the apical ridge (Figure 3.3a and 3.3b),

- Pyriform heads (PEER) where the head narrows in the post acrosomal region (Figure 3.4),
- Nuclear vacuoles(KERN) or the "Diadem defect" which appears as a dark necklace along the anterior edge of the posterior nuclear cap (Figure 3.5),
- Nuclear ridge or fold (VOU) (Figure 3.6),
- Macroephalic heads (MAKR) where the sperm head is larger than normal(Figure 3.7),
- Microephalic heads (MIKR) where the sperm head is smaller than normal (Figure 3.8),
- Abnormal loose heads (ABN) where the head is detached and another abnormality is present (Figure 3.9),
- Double forms (DUBB) which consist of any double whether it be double tails (Figure 3.10) or two heads which is a more uncommon representation of the defect,
- Degenerative head (DEG) where the acrosome is loose.
- Corkscrew midpiece (KRTR) where the midpiece is shaped like a corkscrew (Figure 3.11),
- Stumptails (STMP) this is characterised by a very short stump attached to the base of the nucleus (Figure 3.12),
- Midpiece reflex (MIDS) which is shown by the severe bending of the midpiece (Figure 3.13),
- "Dag defect" (DAG), the tails are either coiled, folded or somehow disrupted (Figure 3.14),
- Broken flagellum (GEBR) where the tails are broken or detached in any way (Figure 3.15),

- Proximal cytoplasmic droplet (PPD) which is when the cytoplasmic droplet is retained in the proximal midpiece position (Figure 3.15) and,
- Pseudo cytoplasmic droplet (PSD) where the cytoplasmic droplet is located near the centre of the midpiece (Figure 3.16).

Minor semen defects are defects that should only be of concern when the occurrence of any of the minor semen defects exceeds 10 – 15% (Blom as cited by Salisbury *et al* 1978) because they are not considered detrimental to the fertility of the semen. The minor semen defects which were taken into account were:

- Normal loose heads (NRM) which is when the sperm head is detached from the tail but there is no sign of other defects on the head,
- Degenerative or loose acrosome (DLA), this defect is considered to be very similar to the major defect, degenerative head,
- Abaxial implantation (ABAK), this defect is characterised by abaxial tails being attached to the head at an angle (Figure 3.17),
- Mitochondrial aplasia (MIT) which is characterised as a fracture in the midpiece (Figure 3.18),
- Curled principle or end piece (KRUL) which is the bending or coiling of the sperm tail (Figure 3.19) and,
- Distal droplet defect (DPD) which is evident when the cytoplasmic droplet is in the distal part of the midpiece (Figure 3.20).

Some of the three categories were calculated and expressed as a percentage

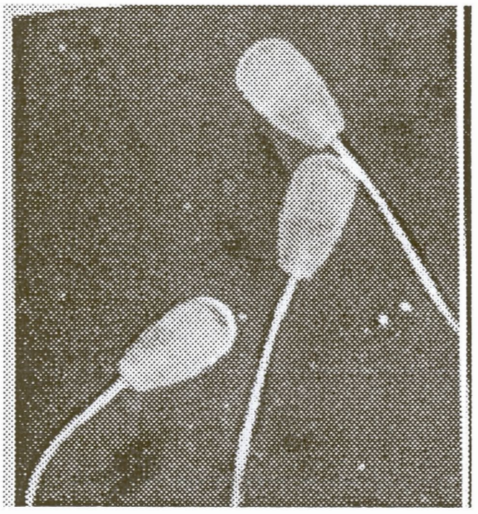

Figure 3.1: Normal bovine spermatozoa*

Figure 3.2: Teratoid forms*

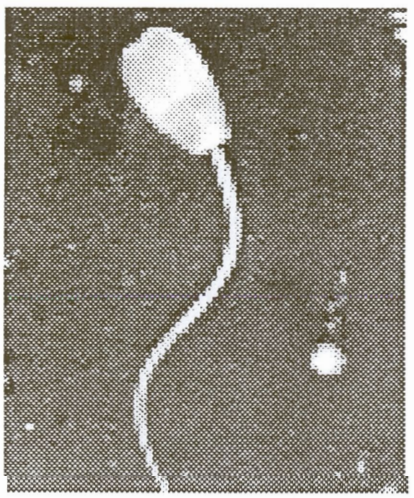

Figure 3.3a: The most common appearance of the knobbed acrosome defect. *

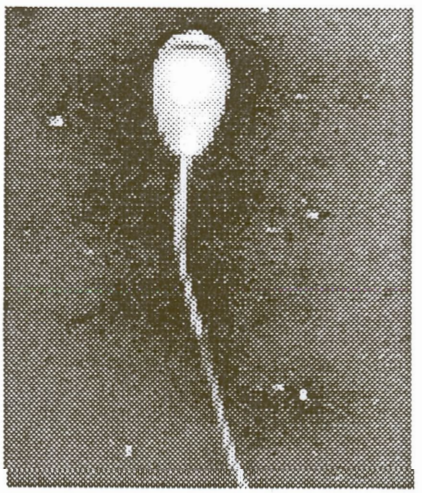

Figure 3.3b: Less common appearance of the knobbed acrosome defect.*

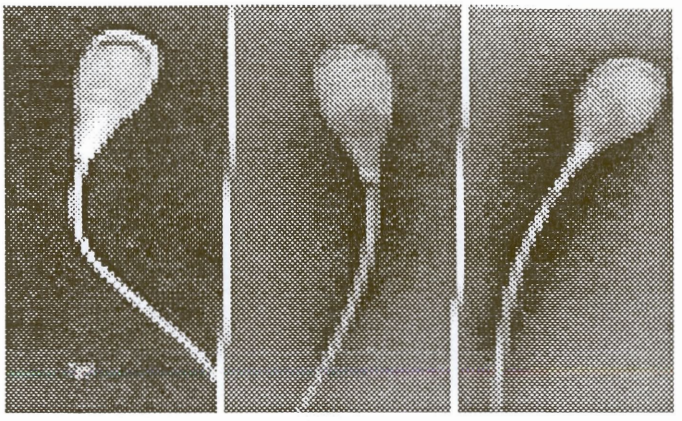

Figure 3.4: Three different forms of pyriform heads which may be observed.*

Figure 3.5: Nuclear Vacuoles in the Equatorial region ("Diadem defect")*

Figure 3.6: Nuclear ridge or fold*

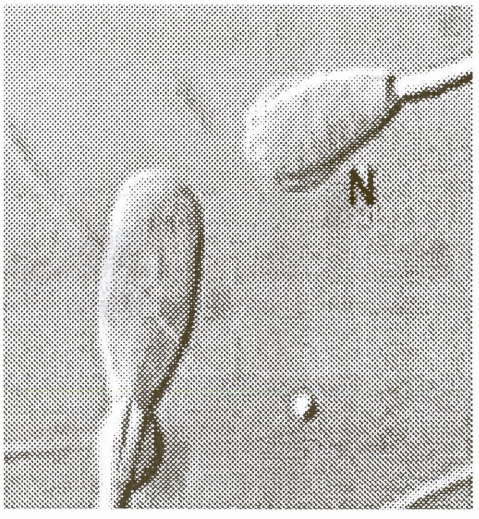

Figure 3.7: Macroephalic head coupled With double tails and the cytoplasmic Droplet defects, N is an example of a Normal normal cell to compare size. *

Figure 3.8: Microephalic head

Figure 3.9: Abnormal loose head.
A detached head with a pyriform base*

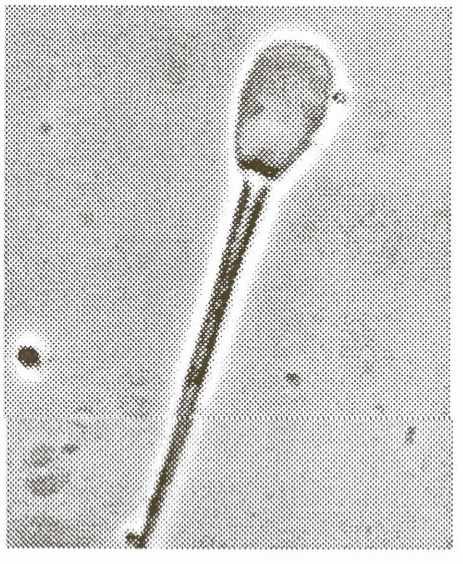

Figure 3.10: Double tails*

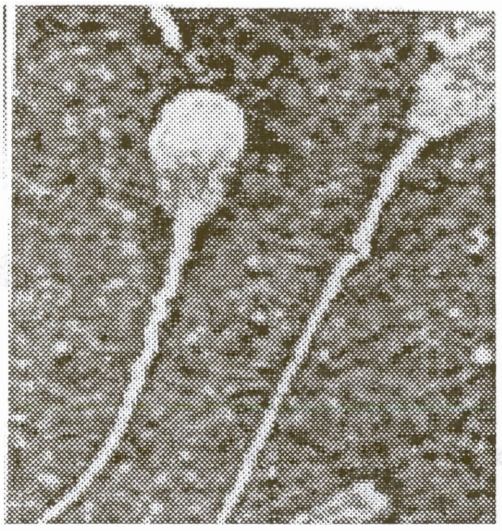

Figure 3.11: Corkscrew defect
Produced from india ink smears.*

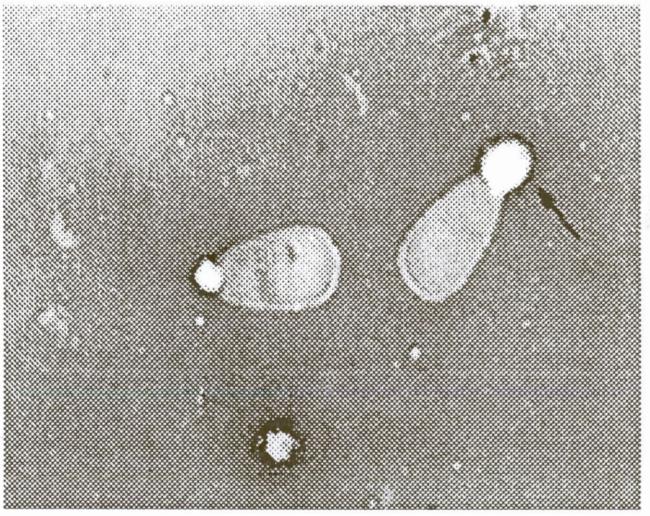

Figure 3.12: Stumptail defect.*

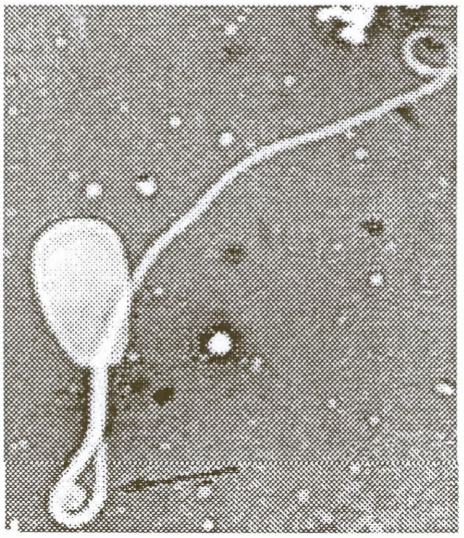

Figure 3.13: A commonly seen form
Of the midpiece reflex abnormality.*

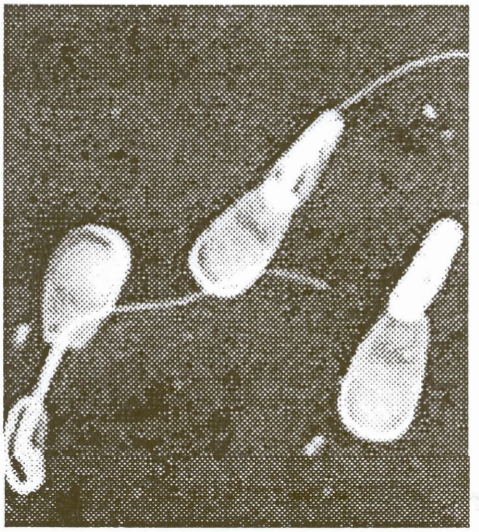

Figure 3.14: The "Dag defect" *

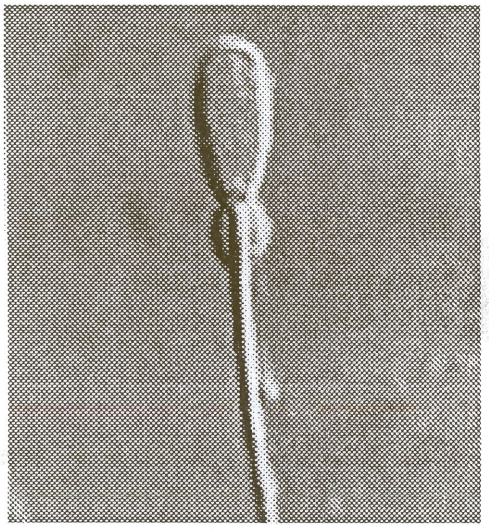

Figure 3.15: Proximal cytoplasmic droplet*

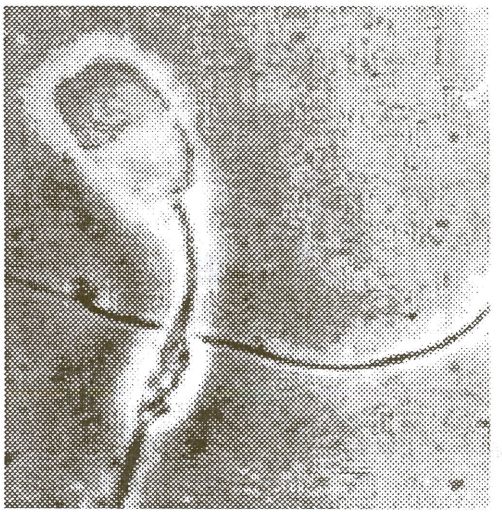

Figure 3.16: Thickening along the midpiece known as the pseudodroplet defect.*

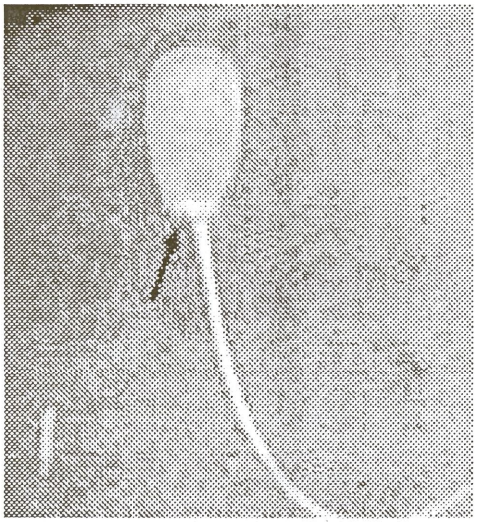

Figure 3.17: Abaxial tail defect

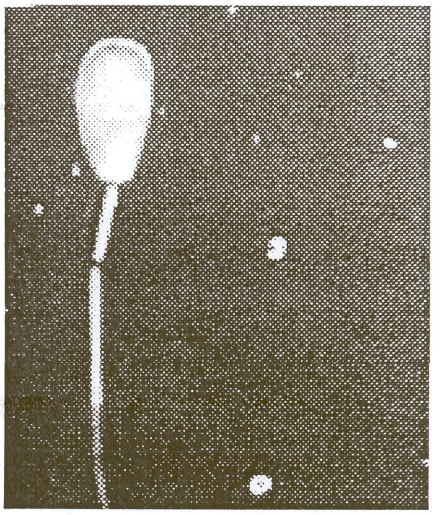

Figure 3.18: Mitochondrial aplasia showing how it can lead to a fracture in the midpiece.*

Figure 3.19: Bent or curled principle piece.*

Figure 3.20: Cytoplasmic droplet in the distal portion of the midpiece.*

(* Figures obtained from Barth and Oko, 1989. Abnormal Morphology of Bovine Spermatozoa)

3.1.2 Statistical Analyses

The effect of vitamin E supplementation and the length of the experimental period were analysed to study the effect of the treatment and treatment period on semen quality and sperm morphological abnormalities over a 90-day period. The data was analysed within and between months but the true effects of the treatment will probably only be evident in the third month after the spermatogenesis cycle has been allowed to complete. The third month is assumed to show the correct effect of the treatment because there may be carry over effects of previous spermatogenesis cycles within the first 60 days of treatment. The results were tabled and were entered into SPSS 11.0 for windows (copyright SPSS Inc. 1987 – 2001) where a GLM multifactor analysis of variance procedure was followed.

3.2 Results and Discussion

3.2.1 General Semen Morphology

Semen quality was evaluated in terms of the percentage normal sperm, while the defects were divided in two categories namely percentage major semen defects and percentage minor semen defects. The effect of Vitamin E supplementation for 30, 60 and 90 days on average percentage normal, major and minor semen defects are summarised in Table 1. The average percentage normal sperm was higher (84,08 ± 11.91%) in bulls fed the diet supplemented with vitamin E compared to those fed the control diet (78,92 ±

11,12%). Both the percentage major and minor defects were lower in bulls supplemented with vitamin E compared to those fed the control diet (Table 1). The effect of dietary vitamin E supplementation on the percentage normal sperm or major and minor semen defects was not statistically significant (Table 1).

Bulls supplemented with vitamin E had 6,13% more normal spermatozoa compared to those in the control group. The supplemented bulls also had 24,12% less major semen defects and 25,9% less minor semen defects when compared to the results obtained from the bulls in the control group. Although statistically not significant, the results do suggest that improvements can be expected subsequent to dietary supplementation with vitamin E. It is known that the bioavailability of the product used was approximately 50%, but it is possible that a higher dose could have yielded more favourable results. A possibility also exists that a more accurate evaluation of the effect of vitamin E supplementation might be possible if more animals were used because in this trial only 4 bulls per group were available. The fact that they are of the same sire reduces the effect of genetic differences, even though the heritability of most male reproductive traits is relatively low, except for scrotal circumference which has a heritabillity of 0,50.

Table 1. **Average percentage of normal, major and minor semen defects of Friesian bulls 30, 60 and 90 days subsequent to dietary vitamin E supplementation.**

	PNORM			PMAJOR			PMINOR		
Treatment	Average	± SD	Sig.	Average	± SD	Sig.	Average	± SD	Sig.
C	78.92	± 11.12		15.92	± 15.64		5.17	± 4.86	
R	84.08	± 11.91	0.444	12.08	± 11.75	0.528	3.83	± 4.15	0.499

PNORM = Percentage Normal Spermatozoa

PMAJOR = Percentage Major Semen Defects

PMINOR = Percentage Minor Semen Defects

C = Control Group

R = Experimental Group

SD = Standard deviation

Sig. = Significance

The significance of the experimental period and the effect vitamin E treatment has on reproductive traits for the 30, 60 and 90 days were examined and were found to be statistically non-significant (Table 2). The interaction between the supplementation of the diet with vitamin E and the experimental period was also examined and was found to be non-significant.

Table 2. **Significance of the summary statistics (average & ± SD) of the percentage normal, major and minor semen defects of Friesian bulls 30, 60 and 90 days subsequent to dietary vitamin E supplementation.**

	Significance		
Defects	Treatment period (P=F)	Treatment (P=F)	Interaction* (P=F)
PNORM	0.941	0.444	0.453
PMAJOR	0.835	0.528	0.503
PMINOR	0.764	0.499	0.482

* Interaction between treatment and time
PNORM = Percentage normal spermatozoa, PMAJOR = Percentage major semen defects, PMINOR = Percentage minor semen defects

The effect of treatment within months (e.g. 30, 60 and 90 days of treatment) was investigated (Table 3) to determine if any significant effects of the vitamin E supplementation were evident on fertility. No significant effects of supplementation were apparent in months 1, 2 or 3, however in month 1 the average percentage normal spermatozoa was higher (85,5 ± 13,33%) in the bulls which were supplemented. In month 2 the average percentage normal spermatozoa in the bulls supplemented with vitamin E was higher in the control bulls (86,5 ± 4,36%) resulting in the supplemented bulls having 7,80% less normal semen than the control group. Presumably the only reason for this slightly lower percentage normal spermatozoa in the supplemented bulls is that the spermatogenesis cycle was not completed, and therefore not able to show the effects of the vitamin E supplementation. This is confirmed by the

increase in the amount of normal spermatozoa in month 3 where the supplemented bulls had 14,37% more normal spermatozoa than the bulls which did not receive vitamin E supplementation. The spermatogenesis cycle in the bull is estimated to be between 56 to 63 days (Bearden & Fuquay 1997), thus it is expected that the effects of vitamin E supplementation on semen quality will only be fully evident in the third month (90 days) of the experimental period. It may have been beneficial to lengthen the experimental period to see if the semen quality improves even more with continued vitamin E supplementation.

The average percentage major semen defects showed a similar trend in the three-month experimental period, with statistically non-significant effects being reported. However, the bulls supplemented with vitamin E showed differences in the number of major semen defects when compared to the bulls that received the control diet (Table 3). In month 1 the supplemented bulls had 30% less major semen defects present compared to the bulls in the control group. In month 2 there were 38,60% more major semen defects in the bulls which received the supplemented diet when compared to the control bulls in the same month. The percentage major semen defects decreased in the bulls supplemented with vitamin E by 31,58% from month 2 to 3 months, but when compared with the control bulls, they had 58,97% more major semen defects.

Table 3. **Summary statistics (average & ± SD) of the percentage normal, major and minor semen Defects of Friesian bulls 30, 60 and 90 days subsequent to dietary vitamin E supplementation.**

		% NORMAL SPERMATOZOA			% MAJOR SEMEN DEFECTS			% MINOR SEMEN DEFECTS		
Treatment	**Date**	**Average**	**±**	**SD**	**Average**	**±**	**SD**	**Average**	**±**	**SD**
C	1	75.75	±	23.26	17.5	±	15.67	6.75	±	7.68
	2	86.5	±	4.36	8.75	±	2.5	4.75	±	2.36
	3	74.5	±	22.99	4.00	±	4.08	21.5	±	23.06
R	1	85.5	±	13.33	12.25	±	11.81	2.25	±	2.22
	2	79.75	±	14.17	14.25	±	15.31	6.00	±	6.68
	3	87.00	±	17.73	9.75	±	10.9	3.25	±	1.89

C = Control group

R = Experimental group

% = Percentage

Similarly, the percentage minor semen defects were lower in the supplemented bulls (2.25 ± 2.22%) than the bulls that received the control diet in month 1. This shows a 66.67% difference in the appearance of minor semen defects between the control bulls and the supplemented animals. In month 2 the minor semen defects increased by 20,83% in the bulls supplemented compared to the control animals. In the third month of the experimental period the percentage minor semen defects decreased by 45,83% in the animals supplemented with vitamin E when compared to the bulls in the control group, the minor semen defects were 84,88% lower in the semen of the bulls supplemented with vitamin E compared to the bulls fed the control diet.

The average values obtained for the percentage major and minor semen defects do however have very high standard deviation values (Table 3). This suggests that the values for both the bulls supplemented and the bulls that received the control diet deviated greatly between the individuals. The small number of animals in the experimental period is most probably the reason for this large deviation in individual values.

From the results presented in table 3, it can be concluded at this stage that there is no significant effect of supplementing the diet of a breeding bull with vitamin E on the semen quality, when compared to bulls that did not receive any vitamin E supplementation which are of similar size, age and genetic composition. Therefore, even though on average there were no statistically significant effects on the percentage major and minor semen defects, a

question was raised as to whether any of the individual semen defects, either major or minor, were significantly affected which could possibly affect the quality of the semen of bulls supplemented with vitamin E.

3.2.2 Major Semen Defects

The summary statistics of the major semen defects for 30, 60 and 90 days subsequent to vitamin E supplementation are summarised in Table 4. Major semen defects have been shown to relate to impaired fertility or to an abnormal condition in the epididymis (Blom as cited by Salisbury *et al* 1978), thus it is important to look at each of these defects individually. The relationship between the experimental period and the major semen defects only showed to have a statistically significant effect on the presence of teratoid (P = 0,05) semen and macroephalic spermatozoa (P = 0,01). The experimental period did not have any significant effect on any of the major semen defects. The effect of the vitamin E supplementation (Table 4) exhibited statistically significant effects on macroephalic spermatozoa (P = 0,02), abnormal loose heads (P = 0,04) and the presence of spermatozoa with degenerative heads (P = 0,02).

The effect on semen quality 30, 60 and 90 days subsequent to dietary vitamin E supplementation in Table 5 shows whether the vitamin E treatment was positively or negatively correlated. In the case of the significant effect of supplementation on the Macroephalic spermatozoa, the bulls which were supplemented with vitamin E, had on average less macroephalic spermatozoa (0,25 ± 0,45%) when compared to the bulls in the control (0,33 ± 0.89%). Therefore the bulls in the control group had 24,24% more macroephalic

spermatozoa than the bulls which received vitamin E supplementation (Table 5). The abnormal loose head defect was positively correlated with vitamin E supplementation, because it was not detected in the semen of the bulls on the experimental diet, but it was present in the semen of the bulls that did not receive any vitamin E supplementation (0,42 ± 0,51%). The occurrence of

Table 4. Summary statistics of the major semen defects of Friesian bulls 30, 60 and 90 days subsequent to dietary vitamin E supplementation

		Significance		
Defect	**Average ± SD**	**Treatment period**	**Treatment**	**Interaction***
Teratoid sperm	1.29 ± 0.488	0.051 *	0.088	0.419
Knobbed Acrosome	3.62 ± 2.79	0.976	0.315	0.832
Pyriform Heads	3.65 ± 3.33	0.904	0.29	0.704
Nuclear Vacuole	9.6 ± 16.23	0.546	0.704	0.375
Nuclear Ridge	1.14 ± 0.378	0.461	0.506	0.892
Macroeph-alic sperm	0.167 ± 0.482	0.010*	0.025*	0.1
Microepha-lic Sperm	2 ± 1.41	0.51	0.222	0.510
Abnormal heads	1 ± ND	0.424	0.042*	0.390
Double Forms	1.14 ± 0.38	0.641	0.506	0.892
Degenera-tive heads	1.63 ± 1.06	0.422	0.017*	0.232
Corkscrew	ND	ND	ND	ND
Stumptail	ND	0.279	0.321	0.161
Midpiece Reflex	2.06 ± 1.12	0.662	0.487	0.688
Other midpiece defects	1.00 ± 0.00	1.00	0.398	0.487
Dag defect	1.17 ± 0.39	0.676	0.222	0.229
Broken Tails	1.00 ± 0.00	0.25	1.00	0.026**
Proximal Droplet	3.57 ± 6.88	0.34	0.43	532
Pseudo-droplet	0.88 ± 0.28	0.615	0.174	0.615

* = Defects which have a significant effect
** = Significant interaction
SD = Standard deviation

spermatozoa with degenerative heads was 20% more in the bulls receiving the vitamin E supplementation (1,25 ± 0,50%), when compared to the bulls in the control group. The standard deviations of these statistically significant effects of treatment show that there is an increased variation between the individuals. The standard deviation values for the above three significant effects were high. In the case of the macroephalic defect and the abnormal loose heads defect the standard deviation value was actually higher than the average percentage of occurrence, this suggests that the occurrence of these defects may deviate greatly between individuals and that there are no or few differences in terms of treatment. As mentioned above the small number of animals in the experiment may be the cause of this large variation between individuals. On average the bulls that were supplemented with vitamin E, had numerically less major semen defects present in their semen than the bulls in the control group.

A major defect not detected in the semen of the bulls, not receiving vitamin E supplementation, was semen with pseudo droplets, but this defect was detected in the bulls that received vitamin E supplementation. The pseudo droplet is located near the centre of the midpiece and appears as rounded or elongated thickenings that contain dense granules surrounded by mitochondria (Salisbury *et al,* 1978).

Blom, as cited by Salisbury *et al* (1978), demonstrated that the pseudo droplet defect was a major semen defect in 5 Friesian bulls and because 2 of these bulls were half brothers a heritable base for this defect could be suspected.

Since no pseudo droplets were present in any of the control animals, the heritability argument is questionable. Blom (cited by Salisbury *et al* 1978)also found that the incidence of affected sperm increases with the age of bulls along with their gradual decline in fertility. This defect also showed a high standard deviation and a low average of occurrence so it may have only occurred in one bull in the supplemented group of bulls.

Table 5. Descriptive statistics and the significance of major semen defects of Friesian bulls 30, 60 and 90 days subsequent to dietary vitamin E supplementation

Defect	C			R			
	Average	±	SD	Average	±	SD	Sig.
Teratoid Sperm	0.58	±	0.79	3.08	±	4.78	0.08
Knobbed Acrosome	1.33	±	1.67	0.25	±	0.62	0.32
Pyriform Heads	2.25	±	2.05	ND			0.29
Nuclear Vacuole	4.92	±	15.48	ND			0.7
Nuclear Ridge	0.42	±	0.51	0.83	±	0.29	0.51
Macroephalic Sperm	0.33 [a]	±	0.65	0.25[b]	±	0.45	0.03[c]
Microephalic Sperm	0.33	±	0.89	0.08	±	0.29	0.22
Abnormal Heads	0.42 [a]	±	0.51	ND[b]			0.04[c]
Double Forms	0.42	±	0.67	0.08	±	0.29	0.51
Degenerative Heads	1.00[a]	±	1.20	1.25[b]	±	0.50	0.02[c]
Corkscrew	ND			ND			-
Stumptail	0.42	±	1.16	0.83	±	0.29	0.32
Midpiece Reflex	1.58	±	1.73	1.17	±	0.83	0.49
Other midpiece defects	0.17	±	0.38	0.33	±	0.49	0.40
Dag Defect	0.75	±	0.75	0.42	±	0.51	0.22
Broken Tails	0.17	±	0.39	0.17	±	0.39	1.00
Proximal Droplet	3.00	±	7.59	1.17	±	1.80	0.43
Pseudodroplet	ND			0.17	±	0.39	0.17

c = a,b differed (P< 0.05) number / average in a row with different superscripts
ND = Not detected
SD = Standard deviation
C = Control Group
R = Experimental Group

Semen with corkscrew (KRTR) midpieces was not detected in either the control bulls or those which received the vitamin E supplemented diets. It would seem that in general vitamin E supplementation lowers the occurrence of major semen defects, but not largely when compared to the bulls which had no vitamin E supplementation.

3.2.3 Minor Semen Defects

Minor semen defects seem to be less important, and they should only be of concern when the occurrence of any of the minor semen defects exceeds 10 – 15% (Blom as cited by Salisbury *et al* 1978). However, the less minor or major semen defects then he better the overall fertility of the bull. The effects of vitamin E treatment on the percentage minor semen defects are investigated in tables 6 and 7.

Table 6. Summary statistics of minor semen defects of Friesian bulls 30,60 and 90 days Subsequent to dietary vitamin E supplementation.

		Significance		
Defects	Average ± SD	Treatment Period	Treatment	Interaction
Normal Loose heads	2.5 ± 2.39	0.861	0.832	0.368
Degenerative loose acrosome	2.78 ± 2.10	0.95	0.316	0.857
Abaxial implantation	2.67 ± 2.08	0.187	0.115	0.187
Mitochondrial aplasia	ND	ND	ND	ND
Curled endpiece	1.00 ± 0.00	0.301	0.521	0.075
Distal droplet	2.70 ± 3.68	0.381	0.608	0.448

ND = Not Detected
SD = Standard deviation

Table 7. **Descriptive statistics and the significance of minor semen defects of Friesian bulls 30, 60 And 90 days subsequent to dietary vitamin E supplementation.**

	C	R	
Defects	Average ± SD	Average ± SD	Significance
Normal loose heads	0.92 ± 2.31	0.75 ± 1.14	0.83
Degenerative heads	2.75 ± 4.27	1.50 ± 0.58	0.32
Abxial implantations	0.67 ± 1.50	ND	0.11
Mitochondrial aplasia	ND	ND	-
Curled endpiece	0.17 ± 0.39	0.08 ± 0.29	0.52
Distal sroplet	0.83 ± 1.03	1.42 ± 3.7	0.61

ND = Not detected
C = Control group
R = Experimental group
SD = Standard Deviationb

The effects of the minor semen defects 30, 60 and 90 days subsequent to dietary supplementation on semen quality show no statistically significant effects of the experimental period or the vitamin E supplementation on any of the minor semen defects (Table 7). The results in Table 6 indicate no significant effects,between bulls fed vitamin E and those nor receiving vitamin E supplementation for minor semen defects. Spermatozoa with Mitochondrial Aplasia were not detected in either of the two groups in the experiment and four of the five remaining minor defects had lower averages in the bulls which were supplemented with vitamin E than the bulls which were fed the control diet with no vitamin E supplementation. It can be assumed that vitamin E supplementation can reduce the occurrence of minor semen defects in the semen of bulls. However, once again there is the presence of high standard deviations, most of which are higher than the average values indicating that these values are unstable and deviated greatly between the individuals.

3.2.4 Effects after 60 days of supplementation

The second month of the experimental period was investigated separately to determine if an increase or decrease in the amount of major and minor semen defects and the increase in the percentage normal spermatozoa in the bulls which received the vitamin E supplementation compared to the same group in the first month of the experimental period had occurred. The results of the percentage normal spermatozoa and the percentage of major and minor semen defects show no statistically significant effects of the supplementation of vitamin E (Table 8).

The same trend was evident as that which was seen in the percentage normal spermatozoa and the percentage major and minor defects for the entire experimental period (Table 3). There was a statistically significant effect found for macroephalic heads (P = 0.05) (major semen defects Table 9). The defect occurred at an average of 1% in the bulls that received the control diet and it was not detected in the bulls that received vitamin E supplementation. The bulls in the control group had more undetected major semen defects compared to the supplemented bulls, this explains the trend which was seen in table 3 for the second month of the experiment. The reason for this however, is not known. An interesting effect was evident, there were some major defects which had exactly the same average occurrence in the control group and the group which had vitamin E supplementation, such as the amount of other midpiece abnormalities and the occurrence of the "Dag Defect". These could be the result of using half brothers and the effect of their age. According to Salisbury, van Demark and Lodge (1978) the occurrence of

midpiece abnormalities increases with age. The "Dag Defect" may be hereditary but it is uncertain. However Blom observed the "Dag Defect" in the semen of two Jersey full brothers.

No statistically significant effects on the percentage minor semen defects were evident 60 days subsequent to supplementation of vitamin E. The average Spermatozoa with normal loose heads and distal droplets had a greater occurrence in the bulls that were fed vitamin E (Table 10). Both Mitochondrial aplasia and spermatozoa with curled end pieces were not present in either the control group of bulls or the bulls that were fed the supplemented vitamin E diet.

Table 8. Descriptive statistics and the significance of percentage normal, major and minor semen Defects in Friesian bulls 60 days subsequent to dietary vitamin E supplementation.

	% Normal Spermatozoa			% Major Semen Defects			% Minor Semen Defects		
Treatment	Average	± SD	Sig.	Average	± SD	Sig.	Average	± SD	Sig.
C	86.50	± 4.36		8.75	± 2.50		4.75	± 2.36	
R	79.75	± 14.17	0.398	14.25	± 15.31	0.505	6.00	± 6.68	0.736

C = Control group
R = Experimental Group
SD = Standard deviation
Sig. = Significance
% = Percentage

Table 9. **Descriptive statistics and the significance of major semen defects of Friesian bulls 60 days subsequent to dietary vitamin E supplement.**

Defect	C			R			
	Average	±	SD	Average	±	SD	Sig.
Teratoid sperm	0.75	±	0.957	ND			0.168
Knobbed acrosome	0.75	±	0.957	2.75	±	4.27	0.396
Pyriform Heads	2.00	±	2.16	5.00	±	5.47	0.347
Nuclear Vacuole	0.75	±	1.5	4.00	±	7.35	0.41
Nuclear Ridge	0.50	±	0.577	0.25	±	0.50	0.537
Macroephalic Sperm	1.00 [a]	±	0.82	ND[b]			0.050[c]
Microephalic Sperm	ND			ND			0.00
Abnormal Heads	ND			0.25	±	0.50	0.356
Double Forms	0.50	±	0.577	0.25	±	0.50	0.537
Degenerative heads	0.50	±	0.577	0.25	±	0.50	0.537
Corkscrew	ND			ND			0.00
Stumptail	ND			0.25	±	0.50	0.356
Midpiecce reflex	1.50	±	1.73	1.00	±	0.82	0.620
Other midpiece defects	0.25	±	0.50	0.25	±	0.50	1.00
Dag Defect	0.75	±	0.96	0.75	±	0.50	1.00
Broken Tails	ND			ND			0.00
Proximal Droplet	1.25	±	0.50	1.00	±	0.82	0.620
Pseudodroplet	ND			0.25	±	0.50	0.356

c = a.b differed (P<0.05) number / average in a row with different superscripts
ND = Not Detected
C = Control Group
R = Experimental Group
SD = Standard Deviation
Sig, = Significance

Table 10: **Descriptive statistics and the significance of minor semen defects of Friesian bulls at 60 days subsequent to dietary vitamin E supplementation**

Defect	Control Group			Experimental Group			
	Average	±	SD	Average	±	SD	Sig.
Normal loose heads	0.50	±	1.00	1.00	±	1.41	0.585
Degenerative loose heads	3.00	±	2.16	1.50	±	1.29	0.278
Abaxial Implantations	0.25	±	0.50	ND			0.356
Mitochondrial aplasia	ND			ND			0.00
Curled endpiece	ND			ND			0.00
Distal droplet	1.00	±	1.41	3.50	±	6.35	0.471

SD= Standard deviation
ND = Not Detected

The third month of supplementation was investigated separately to determine if there were any statistically significant effects once the spermatogenesis cycle had been completed in order to determine the effects of the vitamin E supplementation on the semen. The results in Table 11 confirm the effect that was shown in table 3, there were no statistically significant effects on the percentage normal spermatozoa and the percentage major and minor semen defects 90 days subsequent to vitamin E supplementation. The percentage normal spermatozoa had a higher average in the semen of the bulls that had vitamin E supplementation. Subsequently, the average occurrence of major and minor semen defects were less in the supplemented bulls than in the control group. According to these results, the effect of vitamin E on the semen quality, when measured in terms of the presence of semen defects compared to the control group of bulls, can improve the quality of the semen of A.I. bulls, even if the improvement is only marginal.

Table 11. **Descriptive statistics and the significance of the percentage Normal, Major and minor semen defects in Friesian bulls subsequent to 90 days of dietary vitamin E supplementation**

	C			R			
Defect	Average	±	SD	Average	±	SD	Sig.
PNORM	74.50	±	22.99	87.00	±	10.03	0.357
PMAJOR	21.50	±	23.06	9.75	±	1.90	0.392
PMINOR	4.00	±	4.08	3.25	±	1.89	0.750

C = Control group
R = Experimental group
SD = Standard deviation
Sig. = Significance
PNORM = Percentage normal spermatozoa
PMAJOR = Percentage major semen defects
PMINOR = Percentage minor semen defects

The third month of the experiment showed vitamin E supplementation to have a statistically significant effect on spermatozoa with abnormal loose heads (P = 0,02). Abnormal loose heads were detected in the control bulls at an average of 0,75 ± 0,50% whereas the supplemented bulls showed no evidence of this defect (Table 12). The results on the semen of the bulls fed the vitamin E supplement, indicate that five of the major semen defects that were detected in the second month of the experiment were not evident in the third month. These were Spermatozoa with nuclear ridges, abnormal loose heads, double forms, degenerative heads and stumptails. Thus, resulting in a decrease the percentage of major semen defects and increasing the percentage of normal sperm which increases the fertilising ability of the bulls. The amount of major semen defects which were not detected remained constant in the control group from month two to month three but the type of defect which was not detected changed. The majority of the major semen defects had lower averages of occurrence in the bulls which received vitamin E supplementation when compared to the bulls which were fed the control diet.

Table 12 **Descriptive statistics and the significance of the major defects of Friesian bulls subsequent to 90 days of dietary vitamin E supplementation.**

	Treatment						
	C			R			
Defect	Average	±	Std Dev.	Average	±	Std. Dev.	Sig.
Teratoid sperm	ND			ND			-
Knobbed acrosome	2.00	±	2.16	2.25	±	3.86	0.914
Pyriform Heads	2.00	±	2.83	3.75	±	4.27	0.520
Nuclear Vacuole	13.50	±	27.00	2.00	±	3.37	0.43
Nuclear Ridge	0.25	±	0.50	ND			0.356
Macroephalic Sperm	ND			ND			-
Microephalic Sperm	0.25	±	0.50	ND			0.356
Abnormal Heads	0.75 [a]	±	0.50	ND[b]			0.024[c]
Double Forms	0.25	±	0.50	ND			0.356
Degenerative heads	0.75	±	0.96	ND			0.168
Corkscrew	ND			ND			-
Stumptails	1.25	±	1.89	ND			0.235
Midpiece reflex	1.00	±	2.00	1.25	±	0.50	0.816
Other midpiece defects	ND			0.50	±	0.58	0.134
Dag Defect	0.50	±	0.58	0.50	±	0.58	1.00
Broken Flagellum	0.50	±	0.58	ND			0.134
Proximal droplet	0.50	±	1.00	0.75	±	1.50	0.791
Pseudodroplet	ND			0.25	±	0.50	0.356

ND = Not Detected
C = Control group
R = Experimental group
Sig. = Significance

The percentage minor semen defects showed no statistically significant effects 90 days subsequent to the dietary vitamin E supplementation (Table 13). There was a general decline in the average occurrence of minor semen defects in both the control group and the bulls that were supplemented from month 2 to month 3. Abaxial implantations and mitochondrial aplasia did not occur in the control group or in the experimental group of bulls. The

supplemented bulls had slightly lower averages of minor semen defects when compared to the bulls in the control but were not statistically significant.

Table 13. **Descriptive statistics and the significance of the minor semen defects in Friesian bulls 90 days subsequent to dietary vitamin E supplementation.**

	Treatment						
	C			R			
Defect	Average	±	Std Dev.	Average	±	Std Dev.	Sig.
Normal loose heads	0.25	±	0.50	1.00	±	1.41	0.356
Degenerative loose acrosomes	2.75	±	4.27	1.50	±	0.58	0.583
Abaxial implantation	ND			ND			-
Mitochondrial Implantation	ND			ND			-
Curled Endpiece	0.50	±	0.58	ND			0.134
Distal Droplet	0.50	±	1.00	0.75	±	0.96	0.73

ND = Not Detected
C = Control Group
R = Experimental group
Sig. = Significance

3.2.5 Interactions

The only significant interaction between treatment and the treatment period which was evident in this study was observed for broken tails (p = 0.026) in Table 4. Broken tails were high in the bulls which were in the group that was supplemented with vitamin E in month 1 (30 days) of treatment and the graph (figure 1) shows that the defect was not detected in month 2 (60 says) or month 3 (90 days). In the control group the bulls showed no evidence of broken tails in the beginning of the trial, but after 60 days the occurrence of broken tails began to increase. The bulls in the control had an average of 0,50% broken tails after 90 days compared to 0,0% in the group of bulls supplemented with dietary vitamin E

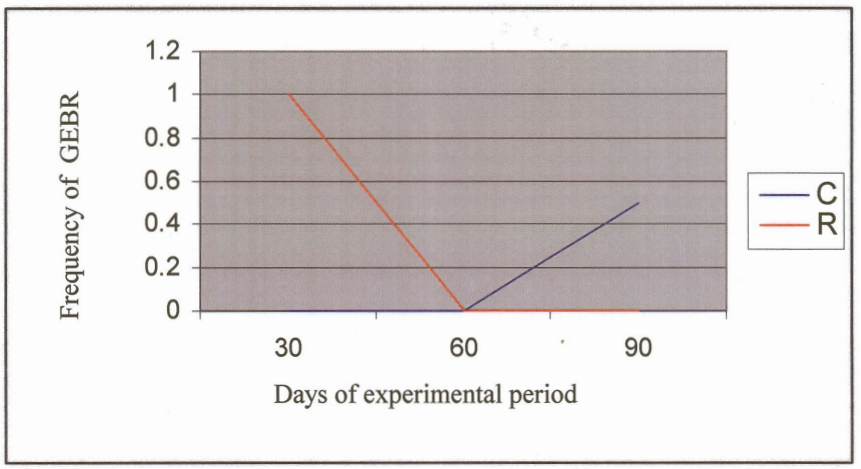

Figure 1: The interaction between treatment and treatment period on broken Flagellum.

3.3 Conclusions

Feeding vitamin E as a dietary supplement does not have a significant effect on the general semen morphology of Holstein-Friesian bulls. Dietary vitamin E supplementation slightly decreased the incidence of head abnormalities, which may improve the fertilising capability of the spermatozoa but the economic implications of the added cost of the supplement is uncertain. Although there was no statistically significant effect of the vitamin E supplementation the results are interesting. It appears that the supplementation can reduce the percentage of major and minor semen defects to a more acceptable level. Although the effects of the vitamin E supplementation on semen morphology were not significant, there may be an effect on the freezability of the semen and the length of time that the semen may be stored. There is very little information on the freezibility and storage

of dietary vitamin E supplementation of semen, with warrants further investigation.

Some of the morphological abnormalities which were present may have been caused by handling procedures and preparation of the semen smears, for example the appearance of bent midpieces may not be due to poor spermiogenesis on the part of the bull but due to a hypotonic solution. The nigrosin-eosin solution used for staining in this experiment was reported by Bishop *et al* (1954) to be hypotonic to bull semen. Swanson and Bearden as cited by Maule (1962) found that if the nigrosin-eosin solution included an isotonic citrate buffer, constant results would be obtained (Maule, 1962). Loose heads may also occur because of breakage in preparing the microscope slides.

The morphology of an individual bull's semen may change from time to time or from season to season, thus it is important to continue monitoring each bull's semen and to examine every collection from bulls that tend to react occasionally to unknown factors with a susequent increase in abnormal cells sperm cells (Salisbury *et al*, 1978).

3.4 Critical Overview

The limited number of animals that were available for this experiment may have had an influence on the results. Understandably the cost associated with obtaining bulls is high and to obtain bulls of similar ages and which are related is extremely difficult, and is therefore a limiting factor. The problem of

having a limited number of animals is that the results of one animal has a significant affect on the average, for instance, in this trial the effect of the performance of each bull constituted 25% of the average per group. A trial with more animals may have reduced the high standard deviations which were seen for most of the effects and more reliable results may have been obtained.

The manner in which semen was collected may have also been responsible for a number of limitations of the experiment due to the procedure being very technical and complex. Although the total number of bulls in the experiment from a statistical point was low, it is difficult to collect semen from so many bulls and it resulted in a stressful environment. Individual bulls react differently to stress and it may have affected the results which were obtained.

The experiment was only conducted in one season (summer), it would have been interesting to test the effects of vitamin E supplementation in other seasons for instance in winter with lower ambient temperatures. The season would also affect the protein and energy levels in the feed, vitamin E may have a greater influence in the absence of these two elements.

This experiment did discover that there is definitely a possibility that the effect of vitamin E in reproduction may be larger than previously thought and I think that it has opened the doors for further investigation, such as, the effect that vitamin E would have on the freezability of the semen. This may have great implications for the artificial insemination industry.

3.5 References

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