

Sveriges lantbruksuniversitet Fakulteten för Veterinärmedicin och husdjursvetenskap Institutionen för kliniska vetenskaper

# Uterine Physiology and Pathology in the Post Partum Period in Ethiopian Cattle

Emma Elisa Källerö

Uppsala

2010

Examensarbete inom veterinärprogrammet

ISSN 1652-8697 Examensarbete 2010:42 SLU Sveriges lantbruksuniversitet

## Uterine Physiology and Pathology in the Post Partum Period in Ethiopian Cattle

Emma Elisa Källerö

Handledare: Hans Gustafsson

Biträdande handledare: Renée Båge, Avdelningen för reproduktion, Institutionen för kliniska vetenskaper

Examinator: Bernt Jones, Institutionen för kliniska vetenskaper Examensarbete inom veterinärprogrammet, Uppsala 2010 Fakulteten för Veterinärmedicin och husdjursvetenskap Institutionen för kliniska vetenskaper Kurskod: EX0239, Nivå X, 30hp

Nyckelord: Ethiopian cattle, involution

Online publication of this work: http://epsilon.slu.se ISSN 1652-8697 Examensarbete 2010:42

## CONTENTS

Summary	
Introduction	
Literature review	6
The post partum period	6
Uterine involution	6
Prostaglandin $F_{2\alpha}$	7
Elimination of bacterial contamination	7
Ultrasound	
Materials and methods	
Animals	
Clinical examinations	
Bacterial sampling and culturing	
Vaginal mucus	
Data analysis	
Results	
Clinical examination	
Temperature	
Cow signals	
Uterine and cervical involution	
Bacterial Sampling	
Vaginal mucus evaluation	
Discussion	
Personal reflections	
Acknowledgements	
References	

#### SUMMARY

The study was performed at a specialized commercial dairy farm; Holetta Agricultural Research Centre (HARC) located 40 km west of Addis Ababa in Ethiopia. Here pure Borana and crossbred Borana x Holstein-Friesian are kept. The postpartum functions were monitored using rectal palpation as well as ultrasonography. The extent of bacterial contamination of the uterus was examined using uterine swabs for subsequent culturing. To obtain a comprehensive picture of the cows' health status traditional clinical examination including examination of the vaginal mucus were used. In addition to this, cow signals were used as a basis for forming a general judgement of the cows' health. The main objectives were to gain deeper knowledge of the uterine pathophysiology during the post partum period in Borana and Borana x Holstein crossbreeds, to compare them with another as well as with high-producing breeds of cattle. The results showed that the uterine involutionary process was similar in Boranas, Borana x Holstein crossbreeds and to that of high-producing breeds. The Boranas presented a higher percentage of uterine bacterial contamination and the Borana x Holstein crossbreeds a lower percentage than that of high-producing cattle. The bacterial findings were similar so the flora found in high-producing breeds. Vaginal mucus evaluation appeared to be an uncertain method for evaluating bacterial findings in the uterine lumen. The results also showed that the body condition during the post partum period was mildly decreased in the Boranas and had increased in the crossbreeds. This is remarkable when comparing with high-producing breeds, which exclusively enters a negative energy balance. Conclusively the low level of production in the Boranas and the crossbreeds protect them from entering a negative energy balance.

## INTRODUCTION

Ethiopia has a population of 74 million inhabitants of which 85% make their living from agriculture. Approximately one third of the agricultural GDP constitutes of livestock production, which renders a cattle population of roughly 43.1 million (CSA, 2008). Small-scale farmers conduct the main part of the livestock production. The majority of the cattle population are indigenous breeds, and among these so-called Zebu cattle, Borana and Horro is the most common. Indigenous cattle are naturally well adapted to the climate but are not as high producing as cows from a more temperate climate. In a study by Lobago et al. (2007) where milk yield data from smallholder crossbred dairy cows in Ethiopia was compiled, the peak yield per day was around 14 kg per day 12 weeks after calving. Therefore wide-ranged crossbreeding between native cattle and high producing breeds such as Holstein-Friesian was introduced in Ethiopia in the 1950's. The raising of native cattle is most common in the Ethiopian highlands, where approximately 80% of the population lives. Crossbreeds are more commonly seen in the lowlands and in urban or peri-urban areas. Pictures of a borana cow and a crossbred cow can be seen in figures 1a and 1b respectively.



Figure 1a. Indigenous Borana

Figure 1b. Crossbreed between Borana and Holstein-Friesian

Milk yield and reproductive efficiency play major roles in determining the profitability of a dairy herd. Factors that influence animal performance are breed, health, management and nutrition (Lobago, 2007).

The period following parturition is known as the puerperium or simply the post partum period. During this time the uterus will undergo a reduction in size and empty itself of bacteria, a process known as the involution of the uterus. In addition to this the ovaries must resume normal cyclical activity. As it is customary to inseminate or mate a cow shortly after giving birth, the aim being one calf born per cow and year, it is important that the puerperium proceeds normally. The current research project is limited within the reproductive field and more specifically to the post partum period, a time associated with multiple disturbances that are decisive for the economy of dairy producers. Numerous studies have been carried out to review the postpartum period in high-producing cows (e.g. Rasbech, 1950; Morrow *et al.*, 1968; Larsson *et al.*, 1984; Kindahl *et al.*, 1992 and Sheldon, 2004), but very few have been made in low-producing breeds like Borana or crossbreeds like Borana x Holstein-Friesian.

In many countries it is common with unnecessary treatments of endometritits and in many cases this is carried out using broad-spectrum antibiotics. The uterus has a powerful self-healing capacity and should preferably be supported instead of treated. It is therefore important to understand the normal pathophysiology of the post partum uterus in order to include a prophylactic perspective so that excessive antibiotic treatments can be avoided.

This pilot study, performed under controlled conditions, will bring fundamental information necessary for future, larger field studies of cows in smallholder farms, and the overall aim is that small-scale farmers in Ethiopia will benefit from the results of the study.

The objectives of the current study are to gain deeper knowledge of the uterine pathophysiology during the post partum period in Borana and Borana x Holstein-Friesian crossbreeds and to compare them with one another as well as with high-producing breeds of cattle.

#### LITERATURE REVIEW

#### The post partum period

#### Uterine involution

After parturition the uterus is grossly enlarged and consequently a reduction in size will follow. The process is known as the involution of the uterus. Its complex nature is due to the placenta in bovines being of a cotelydonary character (Rasbech, 1950). Contraction, tissue repair and loss of tissue are processes that will decrease uterine weight from approximately 9 kg to 1 kg during a 30 days period post partum (p.p) (Rasbech, 1950 and Sheldon, 2004). The involution can be evaluated by rectal palpation and if the involution follows a normal pattern the entire uterus will generally be palpable at day 8-10 p.p. The reduction in weight follows an exponential pattern where a substantial decrease is seen immediately after parturition; 4 days p.p the uterine volume constitutes 50%, and by 8 days 33% of its pregnant dimensions (Rasbech, 1950). The speed of the involution increases by day 10-14 p.p and is probably caused by the uterine contractions that appear at this time. The number of day's p.p when the uterus is considered being fully involuted varies among different studies but both Morrow et al. (1968) and Rasbech (1950) suggests that the involution is completed by day 20-25 p.p. The criteria for a terminated involution are: uterus in a normal position in the pelvic cavity, uterine horns being symmetrical or almost symmetrical and no thickening of the uterine wall. In addition to the uterine changes the cervix will also undergo shrinkage. A decrease of smooth muscle and collagen as well as voiding of fluids causes this. It constricts quickly after calving and by 4 days p.p it will only admit the entrance of two fingers (Noakes, 2009). It takes 3-5 days longer for the cervix and the uterus in an abnormal cow to reach the same size as it does for a normal cow. The uterine involution is illustrated in figure 2. The number of lactations influences the cervical and uterine involution. Multiparae cows ( $\geq 6$ ) have a prolonged involution, which may be explained by the increased uterine size in these animals (Morrow et al., 1969).



Figure 2. Shrinkage of the uterus in a cow following a normal calving (Bekana et al., 1996)

Lochia is a discharge that can be seen up to 18 days p.p. It contains necrotized uterine caruncles and sloughs off in a mixture of blood from the ruptured umbilicus as well as foetal fluids with help from the myometrial contractions. It can be discharged either from the uterus (uterine lochia) or from the cervical canal and the vagina (lochia). Uterine lochia is discharged immediately after calving and contains approximately 1400-1600 ml of fluid. It is initially dark red. After 6-8 days p.p the volume has decreased to 500-600 ml and its character is brown-red and gelatinous. By 12-14 days it is dark red and almost impossible to measure due to its small volume. Uterine lochia contains no slime. Lochia from the cervical canal and the vagina is initially transparent and thereafter changes character to a more chocolate coloured discharge. Before ceasing at day 16 p.p it has a cherry red colour. The total volume is approximately 500-2000 ml. Regardless of origin the lochia should not carry a disagreeable odour. Both uterine lochia and lochia are normal physiologic events during the involution and must not be mistaken for indication of pyometra or metritis (Rasbech, 1950; Morrow et al., 1969 and Sheldon, 2004).

New epithelial cells coat when the caruncles have been sloughed off the endometrial surface. It takes approximately 6-8 weeks before the endometrium is fully regenerated (Sheldon, 2004).

The involution of the uterus is influenced by a number of factors: age, time of year, abnormalities associated with calving (such as dystocia, retained foetal membranes, hypocalcaemia, ketosis, twin births and metritis) and a delayed return to normal cyclic activity in the ovaries (Noakes, 2009).

A good indicator of energy balance can be obtained by approximating the body condition score (BCS). This is of great importance since BCS affects fertility and is correlated to the reproductive performance (Roche *et al.*, 2009)

## Prostaglandin F2.

There are many aspects that influence the course of events during the p.p period and among them oxytocin, prostaglandin  $F_{2a}$  (PG) and the removal of the foetus are important (Noakes, 2009). Among hormones PG plays a key role. In normal cows a long duration of PG release precedes parturition, which will enable a quick involution. PG is believed to have a positive effect on the involution by initiating uterine contractions. On the contrary, in abnormal cows with retained foetal membranes or uterine infection, multiple large irregular amounts of PG will be released and this in addition to the inflammatory process will render a delay in uterine involution. There is a positive correlation between complete uterine involution and resumption of ovarian cyclic activity. Conclusively multiple releases of PG will delay the commencement of ovarian cyclic activity (Kindahl *et al.*, 1992).

#### Elimination of bacterial contamination

The vulva, vestibule, vagina and cervix function as anatomical barriers that protect the uterus from being contaminated by bacteria. These structures enable a normal uterus to remain sterile during pregnancy. During and after calving, however, the relaxed vulva and the dilated cervix allow the entrance of bacteria in to the uterus (Sheldon, 2004). Bacterial contamination of the uterus p.p is common and Swedish studies by Fredriksson et al. (1985) demonstrate that 33% of the animals show bacterial growth during the first week after calving. By the second week the number of cows positive for bacterial culture has increased to 44%. These numbers are in line with Swedish results from Bekana et al. (1996) and English results by Sheldon (2004) but differ from Irish results by Griffin et al. (1974) who claim that the number of cows with a uterine infection during the early p.p period, despite a normal calving, is almost 90%. Though necrotized caruncles, blood and cell debris provides a perfect medium for bacteria to grow, very few bacteria persist and cause metritis or endometritis. In approximately 10-17% of the cows bacteria persist and cause uterine disease (Borsberry & Dobson, 1989). Whether or not the bacteria are eliminated depends on the involution, uterine contractions, endometrial regeneration and defence mechanisms such as leukocyte migration, phagocytosis and inflammatory mediators. Due to this inflammatory response an elevated rectal temperature is often seen in dairy cattle within the first ten days after calving (Sheldon, 2004). Factors that delay the elimination of bacterial contamination are the size of the bacterial load, the character of the bacterial flora, retained foetal membranes (RFM), and depression in the cow's immune status (Sheldon, 2006 and Noakes, 2009).

Common problems among dairy cows are retained foetal membranes (RFM), which may lead to uterine infection. Foetal membranes are defined as retained if they have not been excluded within 24 hours after calving. There are different definitions of uterine infections, i.e puerperal metritis, clinical endometritis, subclinical endometritis and pyometra. Fever, systemic illness and an enlarged uterus discharging watery or purulent fluid characterizes puerperal metritis if clinical signs are detected < 21 days p.p. Clinical endometritis is defined by a purulent discharge in the vagina (>21 days p.p) or a mucupurulent discharge (>26 days p.p), but is not associated with systemic illness. A uterine infection can be defined as a subclinical endometritis when signs of endometritis are absent, but when a cytology sample from the uterus contains more than 18% neutrophiles (21-33 days pp) or more than 10% neutrophiles (34-47 days pp). Pyometra is characterized by an accumulation of purulent material in the uterus in addition to a persisting corpus luteum and a closed cervix (Sheldon et al., 2005). Retention of foetal membranes and or in combination with bacterial infection has proven to delay uterine involution (Königsson et al., 2001). In cows with RFM 100% are positive regarding bacterial findings during the first 3 weeks p.p. This may be explained by the fact that RFM interferes with the uterine contractility and enables bacteria to grow rapidly by providing them a substrate (Königsson et al., 2001). Regardless of having RFM or not, by 7 weeks 100% are bacteriological negative (Fredriksson et al., 1985).

The most common species of bacteria isolated from the uterine lumen p.p are *Escherichia coli, Streptococci, Arcanobacterium pyogenes, Bacillus licheniformis, Prevotella spp* and *Fusobacterium necrophorum*. Among bacteria that are associated with uterine infection (pathogenic species) the most common findings are *E.coli, A. Pyogenes, F. necrophorum* and *Prevotella* species (see table 1).

1	2	3
A. pyogenes	Bacillus licheniformis	Clostridius perfringens
P. melaninogenicus	Enterococcus faecalis	Klebsiella pneumoniae
E. coli	M. haemolytica	Micrococcus species
F. necrophorum	Pasteurella multocida	Providencia stuartii
	Peptostreptococcus species	Proteus species
	Staphylococcus aureus	Staphylococcus species, coagulase negative
	Non-haemolytic Streptococci	α-Haemolytic Streptococci
		Streptococcus acidominimus
		Aspergillus species

*Table 1. Categorisation of bacteria according to their expected pathogenic potential(1-3) in the uterus* 

Table 1 is compiled from data by Sheldon (2005) and the categories are: (1) recognised uterine pathogens associated with uterine endometrial lesions; (2) potential pathogens frequently isolated from the bovine uterine lumen and cases of endometritis but not commonly associated with endometrial lesions; (3) opportunist contaminants transiently isolated from the uterine lumen and not associated with endometritis. (Ruder *et al.*, 1981; Olson *et al.*, 1984; Farn *et al.*, 1989; Noakes *et al.*, 1989; Noakes *et al.*, 1991 and Bonnet *et al.*, 1993). These findings are in line with results from Bekana (1996) and Sheldon (2004). Persisting infection with *A. pyogenes* after 21 days pp will reduce the fertility to first service p.p (Griffin *et al.*, 1974).

When studies have been made to evaluate whether the appearance and odour of vaginal mucus reflects the bacterial findings in the uterus, it has been found that *A. pyogenes, Proteus species* and *F. necrophorum* are related with purulent or mucupurulent vaginal mucus. Furthermore, *A.pyogenes, E.coli*, non-haemolytic streptococci and *M. haemolytica* are associated with a foul mucus odour (Williams *et al.*, 2005). Using a method described by Williams *et al.*, 2005, collecting mucus with a gloved hand and where the hand does not remain inside the vagina for more than 30 s, it has been validated that the examination does not cause a bacterial contamination of the uterus nor prolongs uterine involution (Sheldon *et al.*, 2002)

The bacterial status of the p.p uterus is of great importance since bacteria is known to cause inflammation and damage the endometrium and thereby prolong the uterine involution (Sheldon *et al.*, 2003). Additionally it affects ovarian activity by suppressing pituitary lutenizing hormone secretion and thereby influencing the growth and the function of the follicles (Sheldon *et al.*, 2002).

## Ultrasound

Usage of ultrasound when diagnosing the involution of the uterus is a good technique as it enables quantification and visualization of results. In addition to this the diagnosis of the involution is a far more objective method than rectal palpation. However, if a combination of ultrasound and rectal palpation is used, the involutionary progress admits even more precise results (Okano & Tomizuka, 1987). Furthermore this technique provides quick and non-invasive measure to the inner reproductive organs.

Basic principles of ultrasonography may be explained as high frequency sound waves emitted from a transducer and depending on the tissue these waves encounter, they may either be propagated or reflected to the transducer. Reflected sound waves are then converted to an image on the ultra sound screen. It is the density of the tissue that will determine to what extent the sound waves will be reflected. Dense tissues such as bone or the bovine cervix will reflect almost all of the sound waves and render an almost white image on the screen. Such tissues are referred as hyper echoic. Less dense tissues such as the uterus or the ovaries will reflect the sound waves to a varying degree, which in turn will produce an image





Figure 3a. Ultrasonographic image of a uterine horn

Figure 3b. Ultrasonographic image of a uterine horn with contents in the lumen

of various shades of grey. Figure 3a shows an ultrasonographic image of a nongravid uterine horn in a crossbreed cow. The picture is taken 35 days p.p. The probe is held in a perpendicular angle in relation to the uterine horn, and therefore the horn is represented as a cross-section. The crosses indicate the uterine horn boundaries and measures 1.41 cm. Within the boundaries a dark irregular line can be seen, which confines the uterine lumen. Bovine uterine horns are naturally curled and to the right in the same image another section of the same uterine horn can be seen. Figure 3b is taken from a non-gravid uterine horn in a borana cow 26 days p.p. The crosses demarcate the uterine boundaries and measure 1.23 cm. The lumen is filled with hyper echoic content.

Clear liquids do not reflect the sound waves but simply propagate them and are referred as non-echoic. This will render a black image on the screen and an example of this could be fluid in the uterine lumen or follicular fluid in the follicles of the ovaries. Air reflects the sound waves completely and it is therefore of great importance not only to use gel as a coupling medium but also ensure that the transducer is placed directly on the rectal mucosa as air otherwise will reduce the image quality. The frequency reflects the numbers of vibrations from the sound source per second. When using a low frequency a large area can be investigated due to the sound waves penetrating the tissue, but on the expense of detail in the image. With higher frequency greater detail is accomplished but within a smaller area. Normally a transducer with a frequency between 5.0-7.5 MHz is used when studying the ovaries and the uterus. The transducer is preferably a linear array scanner; transrectal ultrasonography acquires sound waves that are emitted perpendicular to the transducer. The resulting image will be rectangular, where the image of tissues closest to the transducer will appear at the top of the screen (Pierson *et al.*, 1988).

## MATERIALS AND METHODS

## Animals

Experiments were carried out at the HARC, which is a research centre with 231 cows. Here, milk production is pasture-based and prior to calving the cows are put in a tied-up barn. The plan was to use 5 Borana x Holstein-Friesian crossbreeds and 5 Boranas. The first examination was taken out 2-12 days post partum and the cows were examined twice weekly until day 25-46 following parturition. The cows can be seen in figure 4.



Figure 4. Picture of the 10 cows being fed

#### The ten cows are presented in table 2.

Table	2.	Data	of	the	10	dairv	cows
1 0010	<i>~</i> .	Dava	~,	1110	10	cicicity	00110

			Calving (previous) to				
Cow	Breed	Parity	Gestation length	conception interval	Calving interval		
1	Crossbreed	5	273	45	318		
2	Crossbreed	3	274	109	383		
3	Crossbreed	1	271	-	-		
4	Crossbreed	4	277	45	322		
5	Crossbreed	2	277	121	398		
6	Crossbreed	1	280	-	-		
7	Borana	10	276	168	444		
8	Borana	5	277	95	372		
9	Borana	5	275	404	679		
10	Borana	5	275	84	359		

Table 2 shows that the crossbreeds had a lactation number of 1-5, whereas the boranas has a lactation number of 5-10. The length of gestation for the crossbreeds extends from 271-280 days and 275-277 days for the boranas. The biggest difference between the two breeds is the calving interval (days of gestation + number of days between calving to conception), which is 318-398 days for the crossbreeds and 359-679 days for the boranas.

#### **Clinical examinations**

Cows were examined twice weekly and on each occasion the body temperature was measured. Cow signals such as rumen fill, manure, body condition (1-5) and signs of lameness were used to get an apprehension of the general health status of the cow (Hulsen, 2008). Cow signals were scored, signs of heat were registered and the outer genitals were inspected for vaginal discharge at each occasion. By rectal examination the location and consistency of the inner genitals were evaluated. Uterine horns were designated as gravid or non- gravid on the following basis; the gravid horn having the largest diameter on the first occasion of rectal palpation. A picture of a clinical examination can be seen in figure 5.



Figure 5. Clinical examination of a borana cow using ultrasonography at the HARC.

Assessment of the uterine position was made as follows; in the abdominal cavity, partly in the pelvic cavity or in the pelvic cavity. The size of the cervix and the horns were approximated. Thereafter ultrasonography was used to examine the inner genitals more objectively using a real time B mode ultrasound (ALOKA SSD 500) with a 7.5 MHz transducer and a linear array scanner (see figure 5). First the diameter of the cervix was measured. An advance in a cranial direction followed this to the bifurcation of the uterine horns. The horn diameters were measured and their content and endometrial appearance was examined (method according to Bekana *et al.*, 1994).

## **Bacterial sampling and culturing**

Bacterial samples were collected on three occasions from each cow, starting from one-week p.p and with 2 weeks interval. If two bacteriological negative samples were obtained a third sample was not taken from the same individual. Firstly the perineal region was carefully cleaned with soap and water. Secondly a sterile uterine swab (Equi-Vet Uterine Culture Swab, Kruuse, Marslev, Denmark) was introduced into the vagina by parting the labia. The swab was cautiously introduced through the cervix by transrectal manipulation, and when it was clear that the swab was actually in the corpus (confirmed by rectal palpation) the sample was taken. The Equi-Vet swabs contain sterile tops, which are enclosed in a plastic tube. A thicker plastic tube covers the plastic tube and the tops (fig. 6a).



*Figure 6a. Equi-vet tops enclosed in the plastic tube* 

Figure 6b. Equi-vet tops illustrated as exposed during sample collection

The technique for sample collection involves pushing the inner tube through the covering tube; pushing the tops beyond the inner tube and then carefully rotating the tops towards the uterine wall (fig. 6b). Before exerting the swab, the tops is withdrawn within the inner plastic tube, and the inner tube is pulled within the covering tube.

If the procedure is performed correctly there will be no contamination of the sample from the cervix and the vagina. Within hours the samples were cultured on horse blood agar and incubated at  $37^{\circ}$  C. Cultures were inspected for growth after 24 h and after 48 h and pictures of this can be seen in figure 7a and 7b. Bacteria were identified by means of appearance, smell and growth (growth referred to as sparse, moderate or plentiful). The identification was aided by tests to reveal whether the bacteria were positive or negative to indol, lactose and catalase. Some samples were additionally cultured on Edward agar (selective for streptococcus species) and some samples were exposed for TSI (Triple Sugar Iron) and TSIA (Triple Sugar Iron Agar), both used for determining gram-negative bacteria such as *E.coli*.



Figure 7a. Streptococciaea spp growing in a typical chain-like formation on horse blood agar

*Figure7b. Gram-negative E.coli growing on horse blood agar* 

## Vaginal mucus

Once weekly the vaginal mucus was assessed, illustrated in figure 7. Prior to this the perineal region was cleaned thoroughly with water and soap. Thereafter a clean, gloved hand sprinkled with paraffin oil was inserted through the vulva. The outer part of the cervix was examined and thereafter the mucus contents of the vagina were manually withdrawn for inspection. The character of the mucus was scored accordingly: (0) clear or translucent mucus; (1) mucus containing flecks of white or off-white pus; (2) exudates containing  $\leq$  50% white or off-white mucopurulent material; and (3) exudates containing  $\geq$  50% purulent material, usually white or yellow, but occasionally sanguineous. In addition to this the odour of the mucus was scored as (0) no odour; (1) abnormal odour and (2) fetid smelling odour (method and modified scoring system according to Williams *et al.*, 2005).



Figure 7. Vaginal mucus from a Borana. scored (3)

## Data analysis

An inadequate number of newly calved individuals made it necessary to change the original plan and instead use 6 Borana x Holstein-Friesian crossbreeds and 4 Boranas. For the same reason the study only included 6 cows during the first 3 occasions of clinical examination. On the forth occasion the study was complete with ten cows. All cows had calved normally and no cows showed any signs of lameness during the study. One cow, of Borana breed, showed signs of clinical mastitis, which persisted throughout the study. No cow, regardless of breed, showed signs of endometritis.

To give a simplified picture of the results from the clinical examinations, the mean value for body temperature, body condition score, uterine horn size and

cervical diameter have been calculated for each breed on each given occasion. The values of the horizontal axis have been clustered in groups of 3 since there exists a wide distribution of the quantity of days post partum between the cows. This means that each mean value has been calculated from 1 to 6 measurements (crossbreeds) and 1 to 4 measurements (boranas).

Statistic analysis of the results has been calculated using the student's paired t-test.

#### RESULTS

#### **Clinical examination**

#### Temperature

The mean temperature of each breed (crossbreeds and boranas) is presented in figure 8. There were no differences between breeds (P > 0.05).



Figure 8. Body temperature in the 10 cows

#### Cow signals

Rumen fill was constantly 2 for the crossbreeds and varied between 1 and 2 for the boranas. Manure scoring showed a variation between 3-4 for the crossbreeds and 2-3 for the boranas. Body condition score is presented in figure 9.



Figure 9. Body condition score in the 10 cows

#### Uterine and cervical involution

The ultrasound gave an accurate measurement of the uterine horns, but showed discrepancy when it came to measuring the cervix. Therefore the results from the measuring of the cervix were obtained using rectal palpation.

In figure 10, the involution of the uterus can be seen. There were no differences between breeds (P > 0.05).



Figure 10. Uterine horn diameter measured by ultrasonography in the 10 cows

Figure 11 presents the cervical diameters. There were no differences between breeds (P > 0.05).



Figure 11. Cervical diameter approximated by rectal palpation in the 10 cows

#### **Bacterial Sampling**

Results from the bacteriological samplings, executed on 3 occasions, are presented in Table 3.

	Cow 1A	Cow 2A	Cow 3A	Cow 4A	Cow 5A	Cow 6A	Cow 7B	Cow 8B	Cow 9B	Cow 10B
1	Neg	*	Neg	*	∝ haem- strept	Neg	Neg	∝ haem- strept	*	
2	Neg	Neg	Neg	Neg	∝ haem- strept	Neg	Neg	∝ haem- strept	E.coli	Neg
3		С		Neg	С			С	E.coli	С

Table 3. Bacteriological results in the 10 cows (A = Borana, B = Crossbreed) from swabsamples taken on 3 occasions (1 - 3). Asterisk indicates missing samples due to late inclusion in the study and C due to closed cervix. The abbreviation Neg implies negative for bacterial growth,  $\alpha$  haemstrept growth of  $\alpha$  haemolytic streptococcus spp and e.coli growth of Escherichia coli

## Vaginal mucus evaluation

-											
	Cow 1A	Cow 2A	Cow 3A	Cow 4A	Cow 5A	Cow 6A	Cow 7B	Cow 8B	Cow 9B	Cow 10B	-
1	<mark>3</mark> /0	*	1/0	*	<mark>3/2</mark>	*	<mark>3</mark> /0	<mark>3</mark> /0	*	*	-
2	0/0	0/0	1/0	1/0	0/0	0/0	1/0	0/0	1/0	0/0	
3	1/0	1/0	1/0	0/0	1/0	0/0	0/0	1/0	0/0	2/0	
4	1/0	1/0	<mark>3</mark> /0	1/0	<mark>3/</mark> 1	0/0	0/0	1/0	1/0	2/0	
5	1/1	<mark>3/2</mark>	0/0	1/0	0/0	0/0	1/0	1/0	1/0	1/0	
6	0/0	0/0	0/0	1/0	2/0	0/0	1/0	0/0	1/0	1/0	

The results from the evaluation of the vaginal mucus, executed on 6 occasions, are presented in Table 4.

Table 4. Vaginal mucus discharge, character score (0-3) and odour score (0-2) in the 10 cows of crossbreed (A) and borana breed (B). Highest scores (most severe) are highlighted by red and colours respectively. Asterisk indicates missing data

## DISCUSSION

The cows in the study cannot be regarded as being fully representative for the Ethiopian cattle population since the majority of the Ethiopian dairy farmers hold indigenous cattle breeds only. In addition to this there exists a prominent heterogeneity among the cows by means of parity number. Though it would have been most interesting to perform the study at smallholder level this was difficult to implement due to logistic problems as well as financial aspects. Most important however, before evaluating the results is to illuminate the scarce dimension of the material, the study being a pilot. The number of cows was adjusted to the accessible amount of newly calved cows and to what was practically possible to perform during a 6-week period. The bacterial culturing was adjusted to the laboratory limits at the HARC, i.e exclusively aerobic bacteria.

Rumen fill and manure showed no considerable variation regardless of comparing individuals or boranas with crossbreeds. Regarding body temperature, illustrated in figure 8, there were no deviating values of measurement; they all remained within the interval, which is considered being normal body temperature in cattle (38-39.5°C). A statistic analysis showed no significance. However, the body temperature was slightly lower in both breeds towards the end of the study than in the beginning. The cows becoming accustomed to being fixated in the pen may explain this, but more probably it is caused by the subsiding effect of an active immune system, which elevated the body temperature in the first place. The immune system activates proinflammatory cytokines immediately after calving. This increases the rectal temperature and is considered a normal course of events and is not to be misinterpreted as metritis (Sheldon, 2004).

Before analyzing the results of the body condition scoring (figure 9), it is most interesting to study a standard body condition score curve in high-producing cows. Figure 12 illustrates how the body condition starts to decrease when these cows approach calving. Due to the high production of milk and the cows' inability



Figure 12. Body condition score curve in high-producing breeds of cattle

to parry the energy loss by means of food intake, it continues to decrease, a phenomenon that will put the cows in a negative energy balance. It is not before 60 days p.p that the high producing cows will start to increase their body condition. When comparing the standard body condition score curve in highproducing cows with the results from the crossbreeds in the current study, the crossbreeds had not decreased their body condition. Even more surprisingly they had *increased* their body condition 23-26 days p.p. Regarding the results from the boranas, they had decreased their body condition by 0.5 points 40 days following parturition, a reduction, which compared to that of high-producing cows must be considered as mild. An important aspect is that crossbreeds and boranas produce far less milk than high-producing cows. The crossbreeds in this study produced an average milk yield of 9.4 kg per day and the boranas 7 kg per day within 6 weeks after calving. This can be compared with around 40 kg per days 6 weeks after calving in Swedish Holstein cows (Swedish Dairy Association, 2007). Neither the crossbreds nor the boranas are intensely bred, nor optimally fed to enable a high production of milk. Conclusively their low production protects them from entering a negative energy balance.

While presenting great differences concerning body condition score, the involution of the uterus (figure 10) and the cervix (figure 11) seem to resemble that of high-producing cows. Regardless of breed the involution is terminated by 23-25 days p.p, which is in accordance with results from Morrow *et al.* (1968) and

Rasbech (1950). Again it must be stressed that the number of cows in the study is scarce and one must proceed cautiously before drawing into conclusions. Figure 10 illustrates that the gravid horn was initially larger in the crossbreeds than the gravid horn in the boranas. There was also a bigger difference between the size of the gravid- and the non-gravid horn in the crossbreeds than in the boranas. The crossbreeds being naturally larger than the boranas may explain this. The cervical diameter tends to shrink more rapidly in the boranas than in the crossbreeds. The value of P at each occasion of measuring is, however always larger than 0.05 (regarding both cervical diameter as well as uterine diameter) and therefore there is no prevalent statistic significance. In order to obtain statistic significance a larger study is probably required.

Despite the slight dimension of the study it was surprisingly few individuals of crossbreed origin that tested positive for uterine bacterial growth. As shown in table 3 only 1 out of 6 crossbreeds were positive for bacterial growth ( $\propto$ haemolytic Streptococcus spp), which is far less than earlier stated by Fredriksson et al. 1985; Bekana et al. 1996 and Sheldon, 2004. Inability to culture anaerobic bacteria as well as bacteria species requiring special agar may explain this. Another important fact is that not all bacteria samples were collected successfully. On the other hand the low number of cows positive for bacterial growth may be explained by factors such as lack of metabolic stress, routines concerning calving being hygienic and the bacterial load of the herd being low. In addition to this all calvings proceeded normally, there were no cases of dystochia and no cow was presented with RFM. Whereas the number of crossbreeds that presented bacteria in the uterine lumen was low, the opposite was true for the boranas, where 50% tested positive for bacterial growth. One borana was encountered with  $\propto$ haemolytic Streptococcus spp and one with E.coli. The identified bacterial species, regardless of breed, are in line with the most common bacterial findings according to Ruder et al., 1981; Olson et al., 1984; Farn et al., 1989; Noakes et al., 1989; Noakes et al., 1991; Bonnet et al., 1993; Bekana, 1996 and Sheldon, 2004. As can be seen in table 3 as many as 4 out of 6 bacteria samples failed to be executed on the third occasion and it is relevant to question the suitability of using Equi-Vet swabs when collecting bacteria from the bovine uterus. The Equi-Vet swabs are rather thick, which renders difficulties as the cervix continuously contracts following parturition. On the other hand they are non-invasive, in comparison to uterine biopsies. In addition to this they are disposable and there is no need to sterilize the material, which is preferable regarding the circumstances this study was performed under. An important difference when comparing the cows in the study with high-producing cows is that no cow was subjected to persisting bacteria causing metritis or endometritis, as Borsberry & Dobson (1989) stated 10-17% would be. Again, in order to draw far-reaching conclusions a larger study is required.

The inability to culture a complete bacterial flora may also conceal growth of anaerobic bacteria such as *Fusobacterium necrophorum*, which according to Williams et *al.*, 2005 is related with purulent or mucupurulent vaginal mucus. Table 4 shows that 4 out of 6 crossbreeds showed a vaginal mucus character score of 3 on at least one occasion. Half of these showed a simultaneously vaginal mucus odour score of 2. A vaginal mucus character score of 3 was seen in 2 out of 4 boranas on at least one occasion. None of these showed a simultaneously

abnormal or fetid vaginal mucus odour. When comparing the bacterial findings with the character of vaginal mucus both cows contaminated with  $\propto$  *haemolytic Streptococcus spp* showed on at least one occasion, a simultaneously vaginal mucus character score of 3. However, this was also true for 4 out of 7 cows, which were bacteriological negative. The cow contaminated with *Escherichia coli* never produced foul smelling vaginal mucus, which is not in accordance with results by Williams et *al*. Bearing in mind that no anaerobic bacteria was cultured and the number of cows being scarce, using vaginal mucus discharge evaluation as an indicator of bacterial findings in the uterine lumen seems uncertain.

The greatest difference between Ethiopian cattle and high-producing cows from a temperate climate is the level of production. The cows in the study would probably not have presented as good condition nor health or performed so well in the reproductive field if they had been pushed harder to produce more milk. In order to draw far-reaching conclusions concerning the uterine pathophysiologic differences or similarities between Ethiopian cattle and high-producing breeds, a far larger study is required. Given the results of this pilot study it can be stated that the uterine patophysiology in indigenous breeds and crossbreeds is similar to that of high producing cows, but environmental factors such as nutrition, climate and parasites may have a negative effect on their production capacity. The overall solution appears to be to aim at minimizing these negative effects in order to improve the production in the indigenous or crossbred cattle, which in turn will benefit the economy of the small-scale farmer or Ethiopia as a whole.

## PERSONAL REFLECTIONS

Performing research in a developing country demands patience and flexibility. Conditions such as electricity and water facilities may not always be accessible and general circumstances to perform work in may differ a lot from what one is used to. It is not as easy to interpret an image on the ultrasound screen in broad daylight just below the equator, constantly approached by non-fixated cows, as it is in a dark and quiet fixation pen. The attitude towards animals and animal welfare is sometimes impossible to accept and generates anger as well as despair. Language barriers result in misunderstandings, which may lead to confusion and subsequent delays; a person employed to fixate the cows may suddenly not show up one day and the reason behind may not be revealed until a few days later. For the same reasons one might discover by surprise that a cow has calved a few days ago even though one thought one would be informed immediately after the calving. One must understand that high technological equipment such as an ultrasound apparatus or strange instruments such as a bacterial swab may arouse interest and frank demands to have a go. One must accept this unconcerned curiosity, which entails working with an audience of five unknown spectators, some freely expressing their opinions and suggestions for how to do the job. At the same time this curiosity illustrates the general ambition towards development and improvement. Lack of costly equipment has made refined skills only using hands, eyes, ears and nose invaluable for diagnosing, and one can only cherish a sincere admiration when witnessing these proficiencies. Considering the low status of the veterinary profession as it is mirrored in relation to salary in a developing country, it is surprising but also hopeful that students dedicate six years of studies for becoming veterinarians. Finally it is impressive to realize what

incentives lay behind their firm decision to become veterinarians, i.e. to improve dairy farming and in a wider perspective Ethiopia.

## ACKNOWLEDGEMENTS

I would like to express my deep appreciation and thanks to my most charming Swedish supervisors professor Hans Gustafsson and assistant professor Renée Båge for all their unreserved and friendly support. I would also like to thank my Ethiopian supervisors professor Merga Bekana and doctor Fikre Lobago for their help and for introducing me so generously to Ethiopian culture. Thanks to my fellow veterinary students Mande Maeza, Roba Gimbe and Annemo Gångare for great cooperativeness during the field work. Thanks to Elisabeth Bagge at SVA for the bacteriological survey. I am also very grateful to SIDA for the financial support as well as the staff at the HARC. My special thanks go to my brother Erik Källerö for all the help with the statistic analysis.

#### REFERENCES

- Bekana M, Ekman T and Kindahl H. 1994. Ultrasonography of the bovine postpartum uterus with retained foetal membranes. Vet. Med. A. 41, 653-662.
- Bekana M, Jonsson P and Kindahl H. 1996. *Intrauterine bacterial findings and hormonal profiles in post-partum cows with retained foetal membranes*. Acta vet. scand. 37, 251-263.
- Bekana M, Jonsson P, Kindahl H. 1996. *Intrauterine bacterial findings and hormonal profiles in post-partum cows with normal puerperium*. Acta vet. Scand 37, 251-263.
- Bonnett B, Martin S and Meek A. 1993. Associations of clinical findings, bacteriological and histological results of endometrial biopsy with reproductive performance of postpartum dairy cows. Prev Vet Med. 15, 205-220.
- Borsberry S and Dobson H. 1989. Periparturient diseases and their effect on reproductive performance in five dairy herds. Vet Rec. 124, 217-9.
- Central Statistic Authority (CSA), 2008.
- Farin P, Ball L, Olson J, Mortimer R, Jones R, Admey W et al. 1989. Effect of Actinomyces pyogenes and Gram-negative bacteria on the development of bovine pyometra. Theriogenology. 31, 979-989.
- Griffin J, Hartigan P and Nunn W. 1974. *Infection patterns and endometritis during the first seven weeks post-partum*. Theriogenology, vol 1, no 3, 91-105.
- Hulsen J. *Kosignaler*. 2008. Rood Bont Uitgeverij and Vetvice, Holland. Svensk utgåva, Svensk Mjölk.
- Kindahl H, Odensvik K, Aiumlamai S and Fredriksson G. 1992. *Utero-ovarian relationships during the bovine postpartum period*. Anim.Reprod.Sci. 28, 263-269.
- Königsson K, Gustafsson H, Gunnarsson A and Kindahl H. 2002. Clinical and bacteriological aspects on the use of tetracycline and flunixin in primiparous cows with induced retained placenta and post partal endometritis. Reprod. Dom. Anim, 36, 247-256.

Larsson K, Jansson L, Berglund B, Edqvist L-E and Kindahl H. 1984. *Postpartum reproductive performance in dairy cows*. Acta vet. Scand. 25, 445-461.

- Lobago F. 2007. *Reproductive and lactation performance of dairy cattle in the oromia central highlands of Ethiopia with special emphasis on pregnancy period*. Doctoral thesis No. 2007:121, 14-18.
- Morrow D, Roberts S and McEntee K. 1969. *Postpartum ovarian activity and involution of the uterus and cervix in dairy cattle. II Involution of uterus and cervix*. Cornell Vet. 59, 190-198.
- Noakes D. 2009. The puerperium. Noakes D, Timothy J, Parkinson and Gary C. *Veterinary reproduction and obstetrics*. 9<sup>th</sup> ed. (194-205). Saunders, England.
- Noakes D, Till D and Smith G. 1989. *Bovine uterine flora post partum: a comparison of swabbing and biopsy.* Vet Rec. 124, 563-564.

- Noakes D, Wallace L and Smith G. 1991. *Bacterial flora of the uterus f cows after calving on two hygienically contrasting farms*. Vet Rec. 128, 440-442.
- Okano A and Tomizuka T. 1987. *Ultrasonic observation of postpartum uterine involution in the cow*. Theriogenology. Vol 27: No 2: 369-376.
- Olson J, Ball L, Mortimer R, Farin P, Adney W and Huffman E. 1984. *Aspects of bacteriology and endocrinology of cows with pyometra and retained foetal membranes*. Am J Res. 45, 2251-2255.
- Pierson R, Kastelic J, and Ginther O. 1988. *Basic principles and techniques for transrectal ultrasonography in cattle and horses*. Theriogenology. Vol 29: No 1: 3-20.
- Rasbech N. 1950. Den normale involutio uteri hos koen. Nord. Vet.- Med. 2, 655-687.
- Roche J, Friggens N, Kay J, Fisher M, Stafford K and Berry D. 2009. *Body condition score and its association with dairy cow productivity, health and welfare*. J. Dairy Sci. 92, 5769-5801.
- Ruder C, Sasser R, Williams R, Ely J, Bull R and Butler J. 1981. Uterine infections in the postpartum cow: II Possible synergistic effect of Fusobacterium necrophorum and Corynebacterium pyogenes. Theriogenology. 15, 573-580.
- Sheldon M. 2004. The postpartum uterus. Vet. Clin. Food. Anim. 20, 569-591
- Sheldon I, Noakes D, Rycroft A and Dobson H. 2002. *Effect of postpartum manual examination of the vagina on uterine bacterial contamination in cows*. Vet Rec. 151, 531-534.
- Sheldon M, Lewis G, LeBlanc S and Gilbert R. 2005. *Defining postpartum uterine disease in cattle*. Theriogenology, 65, 1516-1530.
- Swedish Dairy Association. 2007. Milk recording data.
- Williams E, Fischer D, England G, Dobson H, Pfeiffer D and Sheldon I. 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the inflammatory response to endometritis in cattle. Theriogenology. 63, 102-17.