Determining the pregnancy rate of artificial insemination and the related factors affecting the pregnancy rate in cattle in the Tanga district, Tanzania.

Name: Hedi Kreeftenberg
Student number: 3993957
Study in context of a Master of Science thesis

Date: 28-8-2020
Institution: Utrecht University
Supervisors:
Dr. F.J.C.M. van Eerdenburg[1], Dr. I. Malele[2], Dr. G. Mbata[2] and Dr. Z. Lyimo[3]

[1] Department of Public Health Sciences, Faculty of Veterinary Medicine, Utrecht University
[2] Vector & Vector-borne Diseases Research Institute, Tanzania Veterinary Laboratory Agency, Tanga, Tanzania
[3] Regional Livestock Advisor, Central Government-Regional Administrative Secretary, Tanga, Tanzania

Abstract
In the Tanga region in Tanzania many farmers rely (partly) upon their dairy cattle as a source of income. Therefore, breeding management is of utmost importance. Cows are polyoestrous and have oestrous cycles throughout the year. Several methods have been developed in order to predict the right moment for breeding. However, some may require extensive access to technology and financial resources. Thus, visual observation of heat is the only option. This is a time consuming and sometimes difficult option, as not all cows show clear signs of heat such as standing heat. In this study a questionnaire was composed in order to answer the question if the adoption of artificial insemination (AI) has a positive influence on the pregnancy rate. 99 farmers from Tanga city council were interviewed about adoption of AI, pregnancy rate, number of inseminations needed for pregnancy and some general factors. However, only 45 interviews were included in the final dataset as a result of excluded non-applicable data points in various variables and interviews.
Using a generalized mixed model, the association between pregnancy rate and breeding system, grazing system and highest education level were tested. Farmers who used AI as a breeding system had a negative association with pregnancy rate ($p<0.05$). Furthermore, all education levels showed significance. In addition, a system to assess the heat detection quality (HDQ) was made. This scoring system included factors such as oestrous behaviour watched for by the farmer, moment and duration of observations, calendar use and doing other work while watching the cows for heat. To the authors knowledge no system to assess HDQ existed before this study. This system can be used by AI technicians and farmers to improve their heat detection quality.

Keywords: Heat detection, Tanzania, conception rate, dairy cattle, artificial insemination

Introduction

Oestrous cycle

Cows are polyoestrous animals, with an evenly distributed amount of oestrous cycles present throughout the year. A typical oestrous cycle lasts 17-24 days (1). This may result in pregnancies year-round. The oestrous cycle consists of two phases; the follicular phase, in which big antral follicles are present and the hormone oestradiol (E2), excreted by the follicles, is predominantly present and the luteal phase in which corpora lutea are present which secrete progesterone (P4) which is the primary hormone in this phase. The oestrous cycle consists of four stages, these represent a subdivision of the two phases. The stages proestrus and oestrus are both part of the follicular phase, metestrus and diestrus are both part of the luteal phase.
The proestrus stage includes the growth of ovarian follicles and oestradiol secretion (2) . During oestrus a cow is sexually receptive which is regulated by gonadotropin-releasing hormone (GNRH) (3).
After oestrus, the luteal phase starts with metestrus, this stage consists of formation of the corpus luteum and the beginning of progesterone secretion. During diestrus there is sustained luteal secretion of P4 by the fully functional corpus luteum. Females in diestrous do not display oestrous behaviour.
For a cow, the ovulation occurs 24-32 hours after the onset of oestrous. The oestrous period lasts 6-24 hours with a mean duration of 8 hours for Holstein Friesian and Jersey cows (2,4). In the process of ovulation not only E2 is involved. During the follicular phase, follicular growth and follicular dynamics take place. Dynamics involve different steps, knowing; recruitment, selection, dominance and atresia. During proestrus, follicular stimulating hormone (FSH) surges result in recruitment of follicles. The preovulatory wave is put in motion, after this FSH drops and concentrations stay low until FSH together with luteinising hormone (LH) result in a peak surge leading to ovulation (1).

Importance of right insemination moment.

Insemination of cows should be done very precisely because a successful fertilisation greatly depends on the insemination-ovulation interval. If a cow is inseminated too early, sperm cannot successfully fertilize the egg because the sperm have aged. On the other hand, if insemination takes place too late, the egg has aged and the chances for a successful insemination have dropped (5–7). Noting that fresh sperm cells have a twice as long lifespan within the uterine tract than frozen sperm, as frozen sperm lasts only 12-24 hours. The lifespan of the cow's ovum is even shorter, 6-12 hours (8). However, the exact time of ovulation can only be predicted and not exactly known. Therefore, all kinds of parameters have been investigated in order to make the best prediction for ovulation time. Nowadays, a series of ovulation time predictors are available such as LH, oestradiol and progesterone concentrations, vaginal resistance measurements, pheromone detection, activity measurements and behavioural oestrous signs (9–14). The latter seems a proper predictor for ovulation time although it is very labour intensive if it is done properly (5).

Based on display of standing heat and mounting behaviour, it was found that ovulation takes place 28-26 (+/- 5(h)) hours after the first display of oestrous behaviour (5). As mentioned before, successful fertilization greatly depends on the insemination-ovulation interval. When determining the most opportune moment for insemination, the quality of the embryo should be considered as well. As poor-quality embryo's result in less than 33% in pregnancy (15–17). For the best quality of embryo's cows should be inseminated 12-24 hours before ovulation (18). However, the best moment of insemination for high quality embryo's might not be the best moment for the highest fertilization rate. A potential fertilization failure might occur with AI performed immediately after onset of oestrus. Although, AI performed after 24 hours after onset of oestrus might result in failure to form an embryo. Therefore, a compromise of insemination 12 hours after the first sign of oestrus was proposed (19).

Situation in Tanga

For long, standing heat was used to determine the presence of oestrus and the ideal moment of insemination(20). However, multiple studies showed that not all cows display standing heat when in oestrus (20–24), Studies show that only a small number of animals (37% and 50%) displayed standing heat as a symptom of oestrus (20,21,25). Furthermore, most oestrous behaviour research has been performed in *Bos Taurus* species. In Tanzania, *Bos indicus* species such as zebu are much more frequent. Through the adoption of artificial insemination, not only zebu but also crossbreds are present in Tanzania. *Woldu et al. (2011)* concluded that indigenous breeds had a lower conception rate than crossbreds (26). Zebu cycles have been studied and in contrast to *Bos Taurus* species, zebu cycles were only 24.4% within the 'normal' range of 18-25 days. About 6% of the zebu cows had a shorter oestrous cycle and 70% had a longer cycle (27). Heat detection is more difficult in zebu cattle as this species is not often in oestrous within this 'normal range'. Therefore, farmers might miss the oestrus period if they only watch for heat during this 'normal range'. (27). Furthermore, repeated mounting was less observed in zebu cattle than in *Bos Taurus* species (28). It was recommended that more time should be spent on heat detection. In combination with the additional use of heat detection aids this should help to increase reproduction in zebu (27).

In the Tanga region in North-eastern Tanzania good heat detection is of utmost importance, as numerous of technologies used for oestrus detection in the western world, such as milk progesterone detection and pedometers to display increase in activity, are not available (29).

Oestrous behaviour scoring system

Van Eerdenburg et al. (1996) developed a scoring system for oestrous behaviour. Oestrous behaviour consists of different behavioural signs, not just the classic standing heat. Although, this sign was scored 100 points and is the best sign as was evaluated by *Van Eerdenburg et al. (1996)* (25). Table 1 shows the scoring system. Although, mucus discharge was frequently used by farmers as a sign of heat, the study carried out by *van Vliet and van Eerdenburg (1996)* pointed out that this is only an indicator if the mucus is clear and produces a long string (>50 cm). Cajoling and restlessness received a low score as cows can display this behaviour for several reasons. However, trained and precise farmers could distinguish restlessness as a sign of oestrus when they are familiar with their cows (21). Assessment of the scoring system showed that mucus discharge was an unreliable oestrus sign as it occurred more often during dioestrus. Restlessness was also marked as an unreliable sign for heat detection (30).Oestrus signs were less observed around periods of milking and feeding as the cows were either being milked or were tied to the feeding fence after milking (21).

Table 1: Scoring system for oestrous behaviour as was composed by van Eerdenburg et al. (1996) (25).

Table 2. Scoring scale for oestrous behaviour.

BEHAVIOUR	POINTS
mucous vaginal discharge	3
cajoling	3
restlessness	5
being mounted but not standing	10
sniffing vagina of other cow	10
resting with chin on other cow	15
mounting (or attempting) other cows	35
mounting head side of other cow	45
standing heat	100

This scoring system is cumulative during a 24 hour period. When observed 12 times per day for 30 minutes, a score of 100 points is reached, the animal is considered to be in heat and can be inseminated if desired. When the cows are observed two or three times per day for 30 minutes, a threshold of 50 points can be applied.

Cows can display oestrous behaviour at any moment of the day and oestrous periods may be short. According to *Van Vliet and van Eerdenburg et al. (1996),* it is beneficial to watch the cows for heat three times a day, after milking and feeding in the morning, in the late afternoon and in the evening (21). A more recent study found 9/12 cows showed signs of oestrus during the night (31). Observation periods should extend 20 minutes to be efficient (21). However, *Heres et al. (2000)* concluded that this schedule was not practical for most farmers as they already had difficulties observing twice daily at the advised times for half an hour. However, the scoring system was valued a good addition to heat detection management (30).

Importance of the present study, what are the benefits?
Some studies found even big potential losses, mostly due to decreased milk production and prolonged calving interval. It was stated that heat detection rates and conception rates have the best effect on decreasing calving interval (32).
Inchaisri et al. (2010) found that a non-optimal reproduction led to economic losses of €34,- up to €231,- per cow per year. Decreased milk yield as well as less pregnant cows were major contributors to the economic losses (32). Losses of this magnitude have a huge impact on the economic well-being of farmers in Tanzania as €34,- is about 92.500 Tanzanian shilling (TZS). This is substantial as one insemination in the Tanga district costs around 20.000 TZS and minimum wage for employees in the agriculture sector is 100.000 TZS per month (33).
The losses are partly through decreased milk yield and are calculated against a 'good' reproductive management system. These numbers might be comparable for European countries. However, losses in Tanzania might be smaller as milk yield per cow per year is lower than for cows in Europe.

Hypothesis
The purpose of this study was to establish insight in factors related to the pregnancy rate in cattle and the use of artificial insemination. Therefore, the following hypothesis has been formulated;
"There is a positive relationship between the use of artificial insemination in cattle and the pregnancy rate in the Tanga district."

The results of this study can be used in order to inform farmers about factors of influence on the pregnancy rate so that with that information, the pregnancy rate can be increased. Which is in favour of the farmers with regards to their economic well-being.

Methods

Composing a questionnaire

A questionnaire was made in order to interview the farmers. This included general questions about the farmer and his or her farm, questions about heat detection and questions about artificial insemination.

While composing the questions about heat detection, steps 4, 5 and 8 of the protocol for successful heat detection were used (29). These steps include information about the moment of heat detection, advice on other activities during observations and monitoring devices. Along with these factors, farmers were asked which oestrous signs they look for in their herd, as not all parameters are equally suitable for heat detection (21).

Based on differences in conception rate between the city and rural areas, the distance from the farm to Tanga city centre was noted as artificial insemination technicians' headquarters and stored semen was stationed there (26). Additionally, farmers were asked about their farming system; what type of grazing system was used as well as if natural breeding or AI was adopted. Furthermore, information about the intervals between first signs of heat detection and moment of insemination was included as well as questions regarding conception rate, insemination number and number of pregnant cows. Last, general questions about highest education level, other jobs and the time spent there were included in the questionnaire.

After completion of the questionnaire, it was pretested on 5 farmers. With the feedback from the pretesting the questionnaires were adapted. Questions about additional devices such as pedometers were left out as these were not used by farmers in the region as these devices needed technical support and were expensive. During the study it became clear that only one farmer had a book with reproduction data available. This was less than expected. Therefore, conception rate could not be included in this study. As a result of this, extra questions were added to the questionnaire in order to try and calculate the pregnancy rates ourselves. Questions about insemination numbers, number of cows bred with in the last year and number of cows pregnant in the last year were used to calculate pregnancy rate. A copy of the questionnaire is included as appendix A.

Description of study area.

Tanga region

The Tanga region, located in the northeast of Tanzania, has different landscapes. In the East it is surrounded by the Indian Ocean, in the North by the border with Kenya and in the West grassland and small hills are present. The biggest city in this region is Tanga city. Within the city and the main roads surrounding the city infrastructure are good, however, once outside the city, many roads are just dirt roads. This is mainly a problem during rainy season as it makes some roads inaccessible to cars.

Tanga region consists of eleven different local authorities of which Tanga city council is one. This last district is further divided into 24 wards.

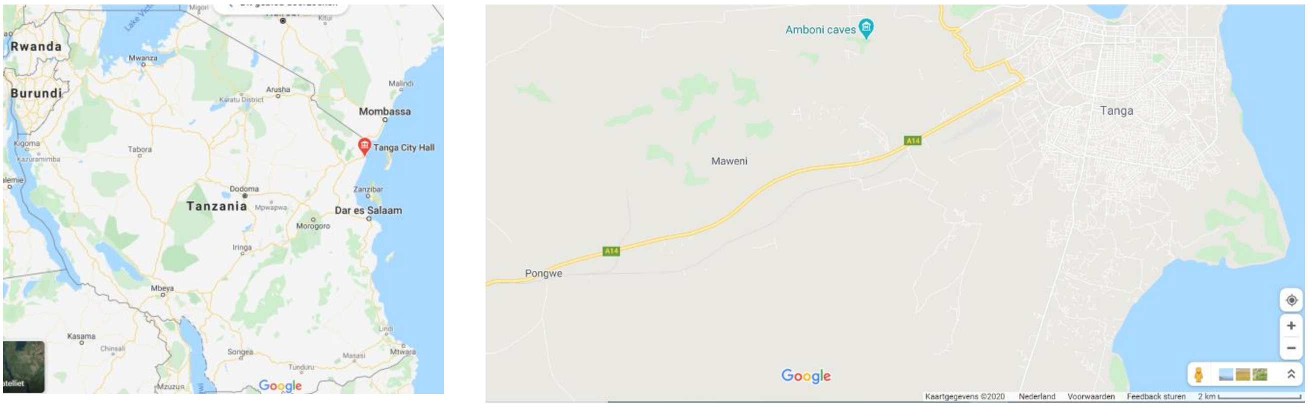

Figure 1: Map of Tanzania with an enlargement of Tanga city. Derived from Google maps

Participant selection

The AI technicians' program ODK collect (PAID-TZN-Service 0020180116) was used to collect a list of all the farmers that use AI in Tanga city council. Which consist of several villages within multiple wards. A list which included a total of 860 farmers was made and after that a sample size of 60 farmers was selected using the random sampling feature within excel. Also, a group of 60 non-AI farmers had been selected as a control group. These farmers were not registered in a system. Non-ai farmers were found by AI technicians asking around. Therefore, this group was not randomly selected.

All completed interviews were put into excel data sheets and transported to R studio version 3.6.1 for statistical analysis. R packages used for this study are: tidyselect, Hmisc, ggplot2, dplyr, stringr, corrplot and lme4.

Explanation about calculation and usage of terminology regarding pregnancy rate.

Terminology

As questions were included to calculate the conception rate, the following terminology was used:

<u>Insemination number</u>: Number of inseminations needed for pregnancy.
<u>Conception rate</u>: Number of conceived cows divided by the total number of services or inseminations. This is different from cows maintaining pregnancy.
<u>Pregnancy rate</u>: Total number of services or inseminations given to a group, in a specified period, resulting in diagnosed pregnancy as a percentage of the total number of services.

However, a true conception rate cannot be determined from a questionnaire. This would rather be the pregnancy rate. Unfortunately, one question was not specific enough to calculate the true pregnancy rate from this. However, it was decided to use this information to calculate the pregnancy rate as there was no proper alternative.

Data inclusion and exclusion criteria

Some farmers owned two farms. For the variable 'number of cattle owned', the total number of cattle owned by the farmer was used for analysis. Combining the numbers of the two farms. Intervals from detection of first signs of heat and calling the AI technician and the interval of calling the AI technician and the insemination were added up. If farmers answered with a range, for example 2-8 hours, the average time was used to categorise and to calculate both the intervals as well as the sum of intervals.

Variables which contained non applicable data (NA) were adjusted. In the final dataset only farms with all questions answered were included. A table was made in order to show the number of missing data per variable.

Determining heat detection quality and development of a scoring system for heat detection quality.
So far, no scoring system for heat detection quality had been developed. In order to evaluate all components influencing the total quality of heat detection a formula was composed to assess the quality of heat detection. Components affecting the quality and, therefore, considered for the formula are; type of oestrous behaviour looked for by the farmer, duration of an observation, moment of the day the observation(s) were carried out, getting other work done while watching the cows for heat and usage of a calendar to predict the next heat.
Adding other oestrous signs and their points to the scoring system, as was conducted by *Van Eerdenburg et al. (1996)*, a total score for oestrous behaviour can be calculated. Points from the other components should be added to this in order to form a total heat detection quality score. If farmers did other work while watching the cows for heat, points for duration were reduced.

Heat detection quality**: (sum of all oestrous behaviour looked for by the farmer expressed in points given by *Van Vliet and van Eerdenburg 1996**) + (sum of points for duration of observation) + (sum of points per moment) – (50% sum of points for duration if other work is done) + (50 points for calendar usage)

* the sum of all oestrous behaviour. For example: The farmer watches for; mucus, restlessness and standing heat. The sum of all oestrous behaviour will be calculated as follows (based on table 1) Sum of all oestrous behaviour = 3 (mucus) + 5 (restlessness) + 100 (standing heat) = 108 points in total.

**"Golden standard" seems to be a 30 min observation without distraction three times a day preferably; morning, late afternoon and evening (21,29,34). In case observation took place only during one moment of the day 'evening and/or night' should receive most points as this period was found to have the most displayed oestrous behaviour as was found by and *McGowan et al. (2007)*, 9/12 cows displayed first oestrous behaviour during the night (31,34).
This resulted in the following scale; points were awarded for the duration and moment of observation. Duration of the observation per moment was given 1 point per minute of observation. If multiple observations were done in one day, de sum of duration of observations was taken. Points per moment were awarded, as evening and/or night was the best moment for heat detection these moments were awarded 40 points. Morning and afternoon were both awarded 25 points. Again, the sum of moments was taken as multiple moments of observations occurred. An extra 10 points were awarded if observations took place more than 3 times a day. In African countries it is not uncommon to use a pastoralist system, in which a herdsman watches over the cows while they graze. Farmers adopting this system received a combined total score of 300 points for duration and moment, as the cows are watched all day long.
It is important to watch the cows for heat without doing other work in the meantime. Farmers who answered that they did do other work while watching the cows for heat, received a 50% reduction on their score for duration. Because their attention was divided between heat detection and other work this will resemble a fair effect in relation to farmers that had all their attention to heat detection. Additionally, if farmers kept record of heat signs and when a cow was supposed to be in heat 50 points were added to their heat detection quality scores.
The maximum score for HDQ is 571 points. 221 points can be scored with oestrous behaviour, another 300 with observation and moment, 0 points reduction because of no other work was done while watching the cows for heat. And an addition of 50 points for using a calendar.

Tables 2 and 3 give an overview of the scores used in this scoring system.

Table 2 :Scoring system for observation moment. Points were given for each part of the day, as evening and night are the best moments to observe these were given the highest score. Farmers who observed more than three times a day were given a bonus of ten points

Moment of heat detection (part of the day)	**Points**
Morning	25
Noon	25
Afternoon	25
Evening	40
Night	40
Observations more than 3 times a day	10

Table 3: Oestrous behaviour scoring system altered after Van Eerdenburg et al. (1996) (25).

Type of oestrous behaviour	**Points**
Mucus vaginal discharge	3
Cajoling	3
Restlessness	5
Raising of the tail	5
Being mounted but not standing still	10
Sniffing vagina of another cow	10
Resting with chin on another cow	15
Colour of vaginal mucus / enlargement of vulva	15
Decreased feed intake	20
Mounting (or attempting) other cows	35
Standing heat	100

Statistical analysis

In order to create an overview of all variables influencing the system of farming, breeding and pregnancy rate a directed acyclic graph (DAG) was made. This graph was also used to check for confounding. After descriptive analysis, a univariate analysis was done in R. Some numbers were rounded and in some cases an average was used in order to run data properly in R. Factors were significant if $p < 0.05$. After univariable analysis with which included information about the mean, standard deviation and correlation. This was tested using Spearman's correlation.

Variables were individually checked for improvement of a multivariable model, using the Akaike Information Criterion (AIC). If the AIC showed an improvement of the model with inclusion of the tested variable it was included in the multivariable model. All variables that did not show an improvement on the AIC score were left out the multivariable model. A generalized linear mixed model was used to test variables for association with the pregnancy rate using the odds ratio (OR), 95% confidence interval (95%CI) and the p-value.

Results

Results are displayed as follows; a descriptive overview of the data set is given following with statistical data specifically about the different breeding systems and a multivariable regression analysis. This dataset showed the results of 99 interviewed farmers along with 62 variables. During the study it became clear that four categories of farmers were present instead of the intended two categories of AI farms and non-AI farms. Two other categories found were mixed system farms which used both AI and natural breeding and drop-out (DO) farms. These farms were supposed to be AI farms as they were collected from the ODK-collect system. They were still registered as an AI farm in this system but no longer used AI services due to various reasons. It was decided to form four categories including the mix and drop-out farms as separate categories.

Descriptive results

Overall factors: In total farmers from 99 farms from 36 villages were interviewed (appendix B). Of all

farmers interviewed, 70/99 were male, 29/99 farmers were female and in two cases both husband and wife managed the farm, and both were interviewed.
In 87 interviews the farmers spoke Kiswahili and the questions and answers were translated. One farmer spoke Kiswahili and a bit of English, the remaining eleven farmers were interviewed in English. In 34 cases farming with dairy cattle was their only job. For 63/99 farmers that were interviewed primary education was their highest level of education (table 4).

*Table 4: Number of farmers per level of education. Scored on their highest level of education. * Mix: In this case both husband and wife managed the farm. They did not attend the same level of education; therefore, they were scored as mix. In one case the combination was no education and primary school. The other combination was primary school and university.*

Highest level of education	Number of farmers (n=99)
No education	1
Primary school	63
Secondary school	14
Vocational	5
College	8
University	6
Mix*	2

Farm factors:
The farms were categorized based on breeding system. 34 farms strictly adopted AI, another 34 farms were AI drop-outs (DO) and 9 farms adopted a mix of both AI and natural breeding. 22 farms strictly used natural breeding (table 5).

*Table 5: Overview of different breeding systems used in the Tanga region.
Combined with on how many farms in the study the breeding system was used.*

Category	Number of farms (n=99)
AI	34
Non-AI	22
AI programme drop-out (DO)	34
Mix of AI and non-AI (mix)	9

Farmers in Tanga adopted not only different breeding systems but also different grazing systems. An overview is given in table 6. The majority of farms used a pastoral system, followed by farms using a mix between pastoral grazing, zero grazing and restricted grazing. Four farms used restricted grazing as their main system. Distances from farms to Tanga city centre ranged between 1 and 33 km. With a median of 10 km and an average of 12 km. Farmers adopting AI lived closer to the city than most farmers that did not use AI (figure 2).

*Table 6: Overview of different types of grazing systems adopted by farmers in the Tanga region. Combined with the number of farms that adopt a certain system. *Several farms adopted a combination of systems. This was often because of different systems were adopted for indigenous breeds and crossbreeds. Plus, some farmers used different systems for calves and adult cattle. However, of the 26 farms that adopted a mixed grazing system 17 farms mixed it with pastoral grazing.*

Farm system type	**Number of farms (n=99)**
Zero grazing and tied up	20
Restricted grazing	4
Pastoral	49
Mix *	26

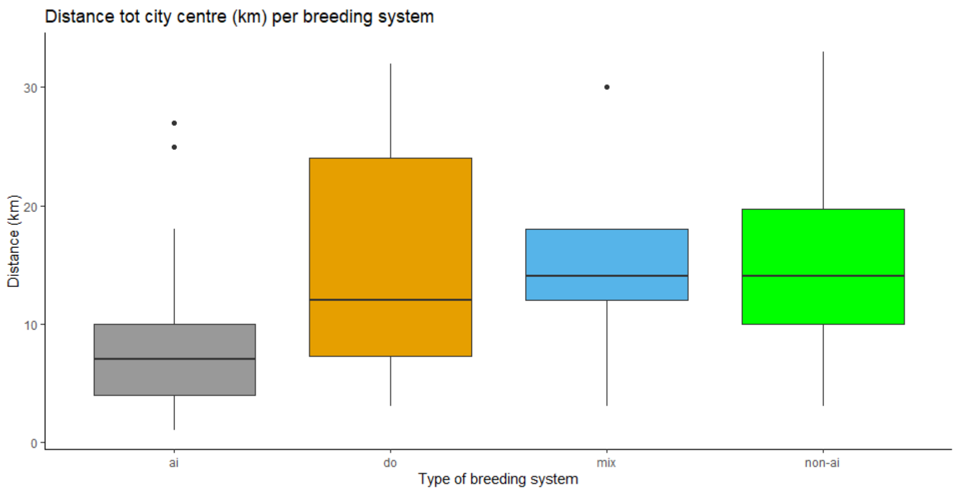

Figure 2: Distance of farms to Tanga city centre in kilometres (km) plotted against type of breeding system.

The minimum number of cows owned was 1, maximum was 900. The mean is 24 cows, however most farmers owned 7 cows. An overview of number of cows per breeding and grazing system is given in figure 3. Most farmers milked 3 cows at the time of questioning. Mean number of cows being milked was 5. Some farmers milked no cows at that moment. However, one farmer milked 210 cows. The most money per litre of milk was earned by farmers in the AI group (figure 4). 1400 TZS/L (€ 0,51) was the highest payment recorded. Mean amount of money made per litre was 900 TZS (€0,33). Most of the farmers within the AI group make more than 900 TZS (€0,33) per litre (figure 5). Overall, most farmers earned 750 TZS (€ 0,27) per litre milk. Some farmers in the non-AI group received as little as 600 TZS (€ 0,22).

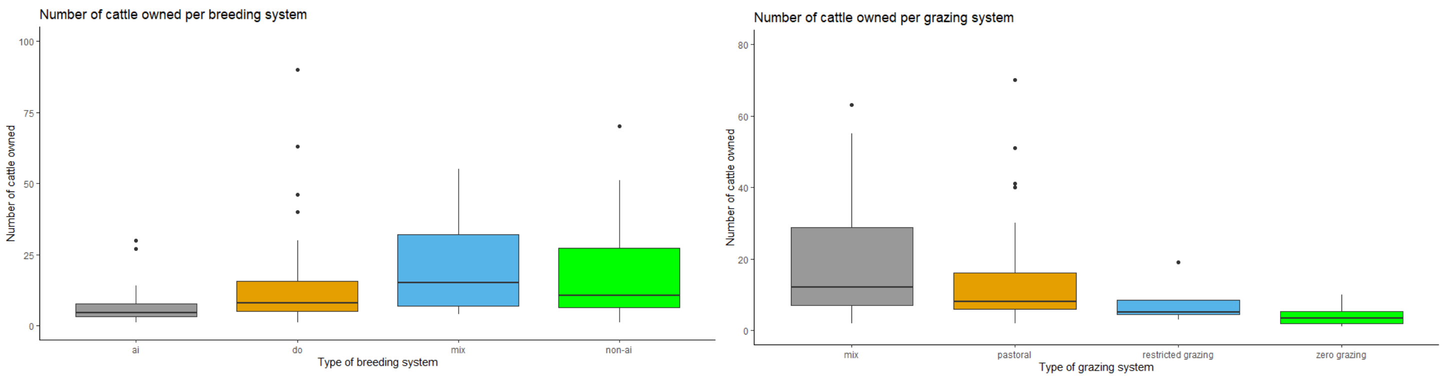

Figure 3: Number of cattle owned per breeding system (left) and per grazing system (right). This figure is altered to fit to scale. Two datapoints were removed from the image as they were very large. Two non-ai farms owned a large amount of cattle (900 and 194 animals). The largest herds are owned by farmers not adopting AI. However, those two data points are not shown in this figure.

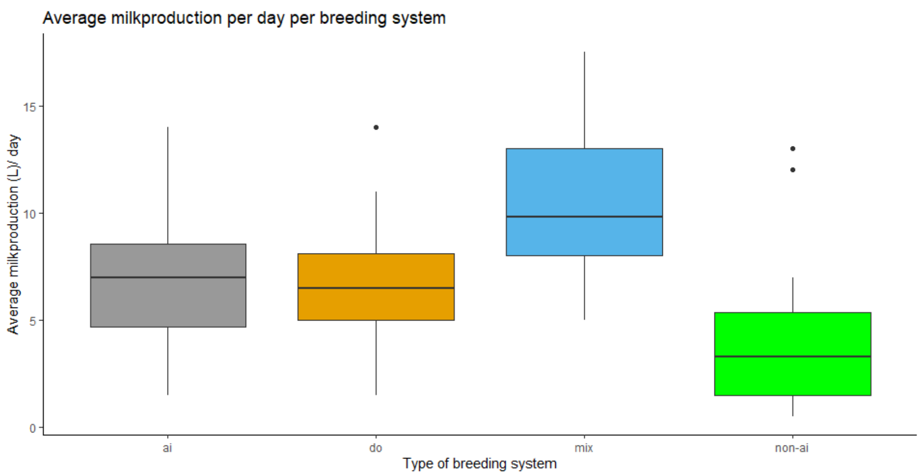

Figure 4: Average milk production per day in litres. Displayed per breeding system. ai = Artificial insemination, do = drop-outs, mix= both ai and natural breeding are adopted, non-ai= only natural breeding is used.

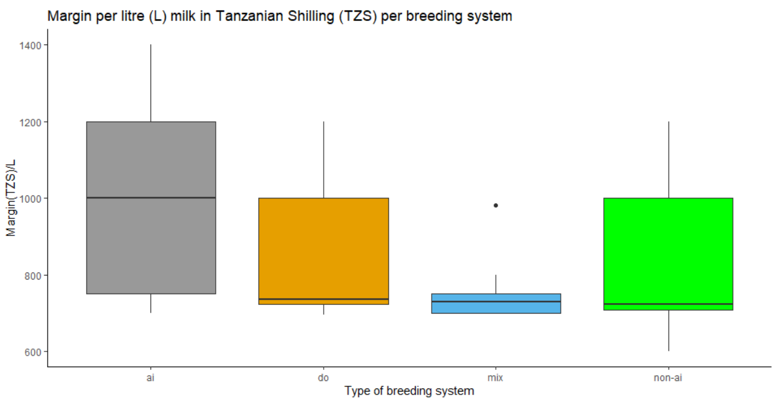

Figure 5: Selling price per litre of milk in Tanzanian Shilling (TZS) plotted against breeding system.

General AI factors: Out of the 34 farmers that adopted AI at that moment only one said that he might use a bull for natural breeding later. However, 16 of those farmers owned a male calf at the time of the interview. Most of them were not to be used as a breeding bull in the future but instead they were to be sold. Of all farmers only 21/99 did no other work while watching the cows for heat. To the question if AI technicians manually check the cow for heat 28 said that the AI technicians check this before inseminating the cow. 1 farmer said that the AI technician does not check this. Table 6 and table 7 give overviews of farmers' choices in AI technician and the used bull.

Table 6: Overview of farmers choice in AI technician

Choice in AI technician	**Number of answers (n=34)**
Yes, I choose	25
Yes, I choose (based on availability)	1
No, there is only 1 AI technician available	3
No, the company or the project chooses	5

Table 7: Overview of choice of farmers in which bull would

Choice in bull (breed)	**Number of answers (n=34)**
No, farmer cannot choose	28
Yes, the farmer can choose	6

Table 8: Overview of scores for observation moment. 300 points were given for farmers who watched the cows all day when they grazed. This score for the pastoral system is the combined score for moment and duration

Points for moment of heat detection	Number of farmers (n=99)
0	9
25	5
40	3
50	8
65	36
75	4
90	15
100	4
105	3
300	10
unknown	2

Heat detection factors:
For the behavioural signs watched for by farmers, some said to be looking for 'increased activity, more noises, cow is more alert or raising ears', all these factors were part of the behavioural sign 'restlessness'. Therefore, restlessness was scored once. There were 6 possible answers in our questionnaire that were not in the scoring system of *Van Vliet and Van Eerdenburg (1996)*. However, points could be given to the signs 'decreased feed uptake and colour and enlargement of vulva(mucus)' (35). These signs were added to the scoring system as is shown in table 3. Twenty farmers said they looked for a drop in milk yield as a sign of oestrus. This sign was not included in the scoring system.
One farmer told us he watched for standing heat amongst other things, however, the translator clearly told him to say this. Therefore, this sign for this farmer was not considered when calculating the total oestrous behaviour score. Table 8 shows the total points for moment of heat detection. Scores per moment are shown in table 2.

Only 12% (12/99) of the farmers told they used a calendar. Points for duration were reduced by 50% if any other work than watching the cows for heat was done in the meantime. 37% of the farmers answered that no other work was done in the meantime. In some cases, no information was present on the signs watched for by the farmer. Therefore, these cases scored 0 points on 'total points for oestrous behaviour'. The highest score obtained in this segment was 143 points, with a median score of 43 points and a mean of 44.05 points.
Farmers were asked if they followed training in heat detection. Table 9 shows an overview of the number of farmers that did and did not follow heat detection training. It was also stated which professional led the training. Half the farmers did not follow any heat detection training.

Table 9: Overview of farmers who followed heat detection training and which profession led the training.

Followed training, given by	Number of farmers (n=99)
Yes, trained by a veterinarian	16
Yes, trained by an AI- technician	9
Yes, trained by other farmers	1
Yes, trained by both a veterinarian and AI-technician	26
No	47

The factor heat detection quality (HDQ) was plotted against type of breeding system. Overall, the drop-out farmers had the best mean HDQ score as well as the biggest variation. Farmers who used natural breeding as their choice of breeding system had the lowest mean HDQ score (figure 6.)
The lowest score obtained was 0 points, the highest score obtained was 488 points. The mean score was 159.8. The highest achievable score is 571 points.

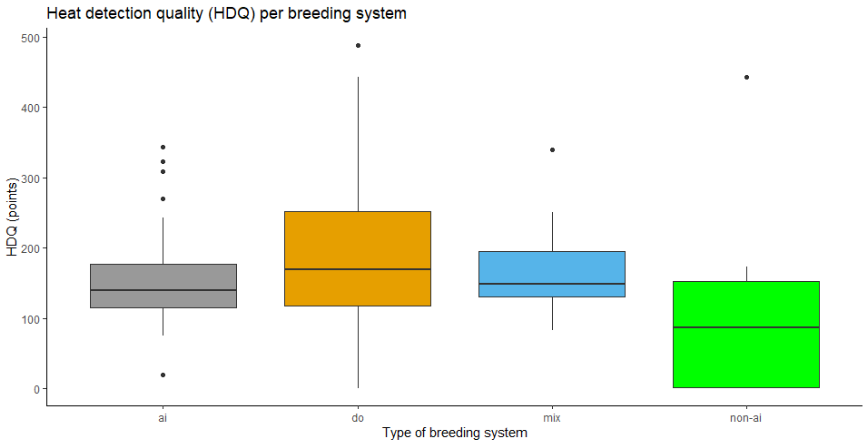

Figure 6: Heat detection quality (HDQ) in points per breeding system.

Intervals:
The total interval time between first signs seen during heat detection and the AI technician inseminating the cow resulted three times in an 'unknown' maximum time. This as a result of farmers telling that when the AI technician was notified about the cow in oestrus, the AI technician himself or herself choose the moment of insemination. One English speaking farmer told that sometimes AI technicians showed up within two hours after calling, however, sometimes they never showed up. Table 9 shows that in 15% (5/34) of all cases insemination takes place more than 12 hours after the first signs of heat.

Table 3: Overview of different intervals expressed in categories of length in hours. IHDC= Interval first heat detection and calling of AI technician. ICAI = Interval calling AI technician and insemination. IHDI= Interval heat detection and insemination. IHDN = Interval first heat detection and natural breeding. IHDB= Interval heat detection to breeding. This includes both breeding through AI and natural breeding. AITD= AI technician decides. Un= Unknown

IHDC	Number of farmers (n=45)	ICAI	Number of farmers (n=45)	IHDI	Number of farmers (n=45)	IHDN	Number of farmers (n=59)	IHDB	Number of farmers (n=104)
<1	27	<1	9	<2	2	<1	39	<1	34
<8	9	<2	10	<3	5	<4	2	<2	2
<10	2	<4	5	<5	4	<6	6	<4	7
<12	1	<6	2	<7	2	<8	2	<6	9
< 16	1	<8	5	<9	8	<10	2	<8	4
>12	1	<10	3	<10	3	<12	4	<10	13
>24	1	<12	3	<11	5	<14	1	<12	10
Un	3	<16	2	<12	1	Un	3	<14	5
		<18	1	<13	3			<17	2
				<14	1			Un	11
		AITD	3	<17	2				
		Un	2	Un	9				

Pregnancy rates

Out of the 34 farms that adopted AI, the pregnancy rate was calculated for only twenty farms. One farmer with two farms scored a PR of 400 upon first calculation. However, the number of inseminations for the ten cows on the first farm was unknown. Therefore, upon second calculation only the number of cows and number of inseminations of the second farm were considered. Farms that used natural breeding were asked how many services from the bull were needed for pregnancy. With this information the pregnancy rate could be calculated for 50% of the non-AI farms (11/22). Out of the twenty pregnancy rates calculated for farms adopting artificial insemination, seven AI-farms and four non-AI farms managed to have a 100% score. Because 35% of the AI- farmers and 36% of the non-AI farmers have scored this maximum score the third and fourth quartile of the boxplot have the same value (figure 7). Out of all farmers in 29 cases the PR could not be calculated as specific questions needed for calculation were incorporated in the questionnaire after interviewing these farmers. For another seven farmers it was impossible to calculate the PR as well due to the fact that the farmer did not know how many inseminations or services from the bull were needed for pregnancy or that the cows he bred with that year still had to be checked for pregnancy. One farmer said they had not bred with any cows in the past year. Therefore, for only 63/99 farms the PR could be calculated.

In all breeding systems there are farms present with a 100% PR. Most systems have a high PR. However, the group that uses AI has the lowest median of all groups and a bigger interquartile range than the other groups. Displaying a wide variety within the group. Some farmers within the AI group had pregnancy rates as low as 0%.

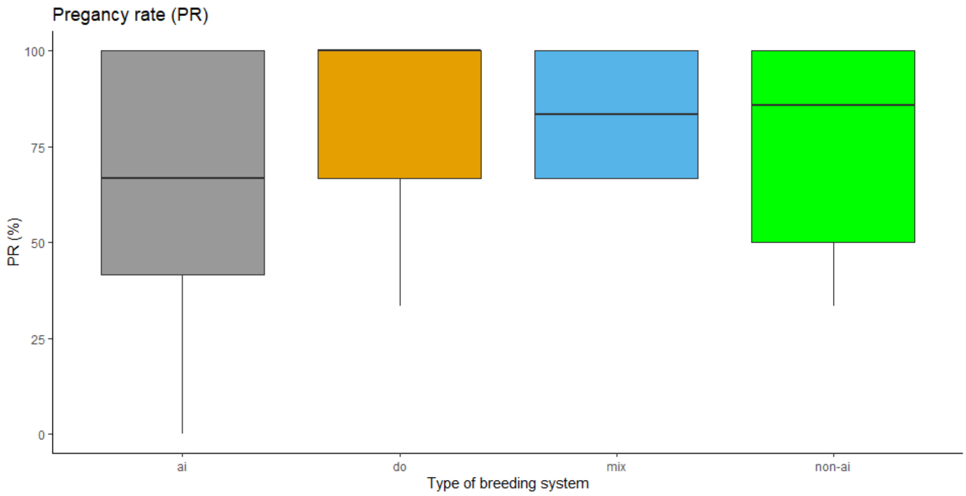

Figure 7: Boxplot of calculated pregnancy rate in percentage (%) for all four breeding systems.

Plotting PR% against type of grazing system it was observed that all systems have high pregnancy rates. However, farmers using zero grazing have the highest variance within their score. Some farmers scored less than 50% on their PR score. The median of farmers using a mixed grazing system is lower than the 50% PR mark. Thus, more than 50% of the farmers using this mixed grazing system score less than 50% on their pregnancy rates (figure 8). For an overview of PR, breeding and grazing system see appendix D.

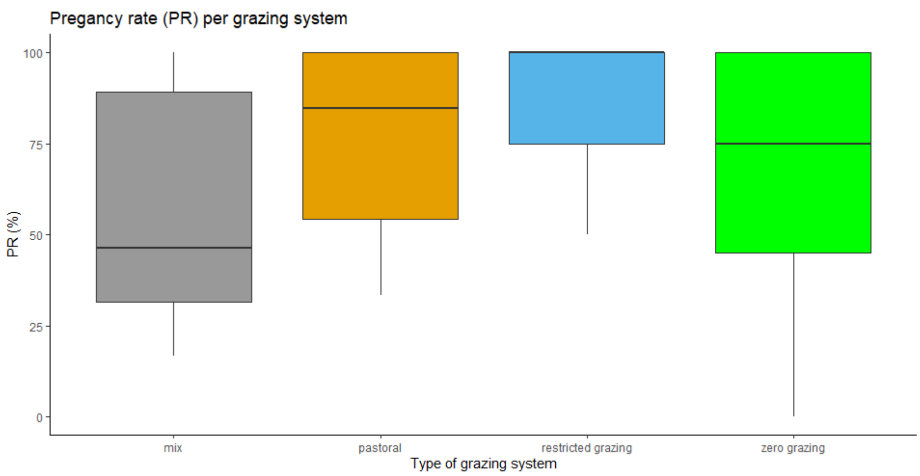

Figure 8: Boxplot of the pregnancy rate (PR) in percentage (%) plotted against the type of grazing system that was used.

A 100% pregnancy rate is interesting as all farmers were asked how many inseminations or services from a bull were needed on average to get a cow pregnant. The mean inseminations needed was 1.9. Excesses of 13 inseminations were also seen. Implying that a PR slightly lower than 100% could be expected.

Figure 9 shows the PR plotted against the distance to Tanga city centre. It shows that most farms, of which the PR could be calculated, are located within 15 km of Tanga city centre. It also shows that all farms with a PR lower than 25% are located within a range of 10 km of the city centre. Furthermore, farms with a PR lower than 60% are located less than 15 km from Tanga city centre (figure 9).

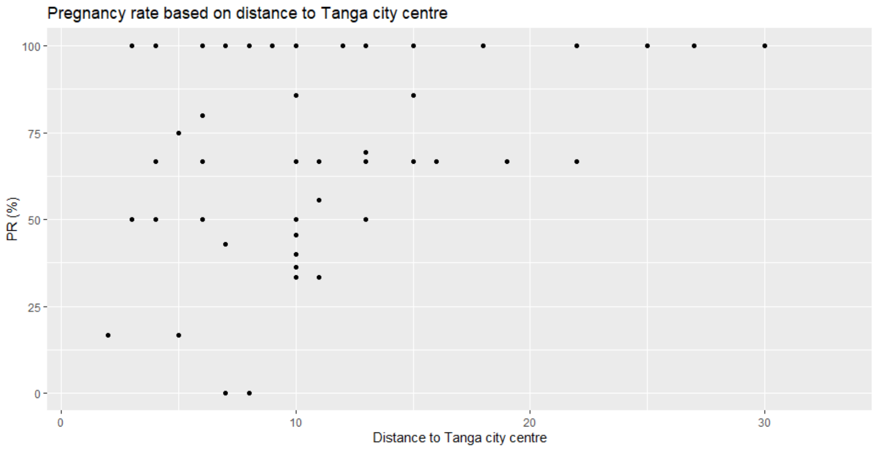

Figure 9: Pregnancy rate plotted against distance to Tanga city centre. Every dot represents one farm. Not all farms are represented in this scatterplot. Only those farms of which the PR could be calculated (63/99) are represented here.

This system is a complex system with multiple variables possibly influencing the pregnancy rate. In the DAG showed in figure 10 it can be seen that multiple variables might have an influence on the PR. Not all of which were included in this study. Pregnancy rate being the outcome variable of this study and type of breeding system, such as AI, the exposure. This schedule is made to check for confounding. In this case the interval between heat detection and breeding might be an effect of the type of breeding system. Therefore, it cannot be a confounding factor.

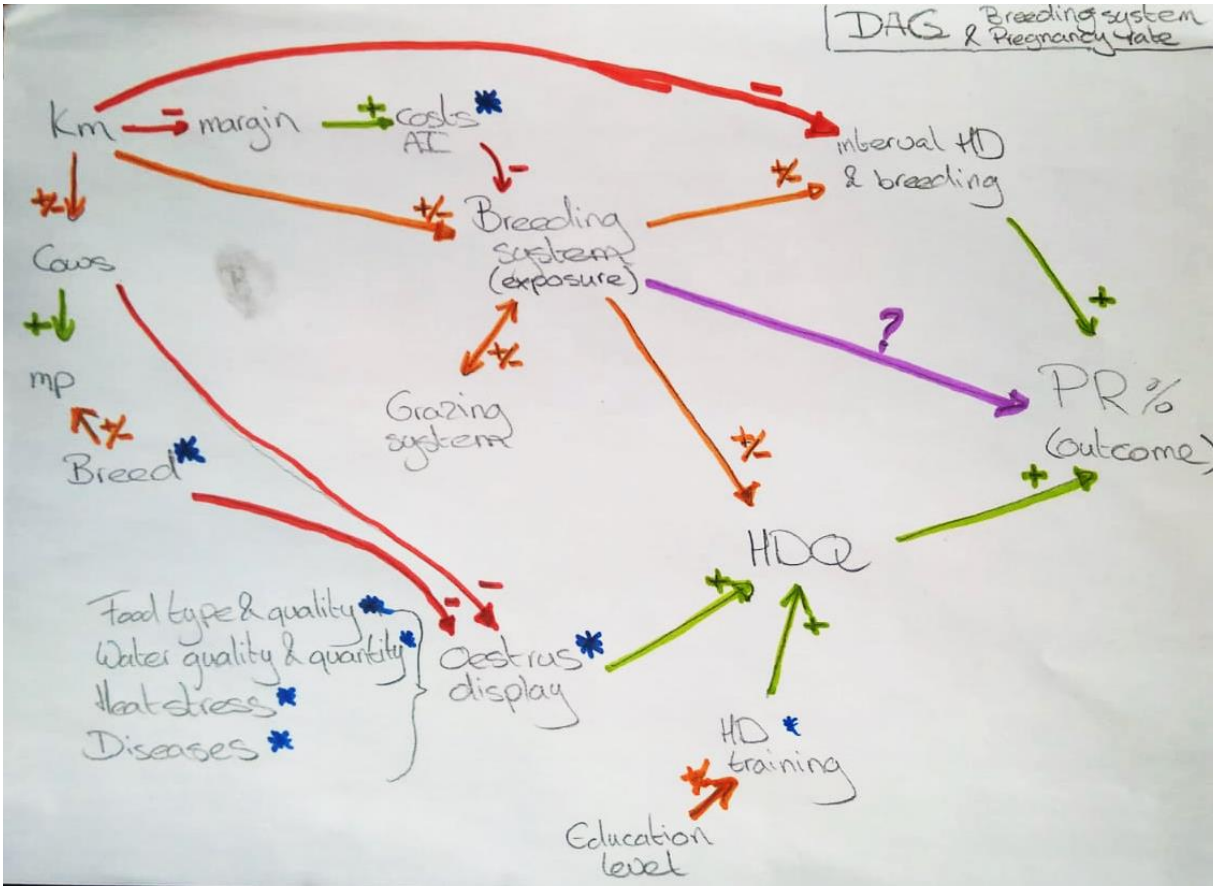

*Figure 10: DAG diagram of all factors involved in relation to the type of breeding system and the pregnancy rate. Colours are indicators for the expected impact. Red for negative, green for positive and orange for a neutral or the effect is expected to have a positive or negative effect depending on the outcome of that variable. * (in blue) these factors were not used in further analysis either because they were not in the questionnaire therefore no data is available or for costs of AI it is known how much AI costs. However, prices of natural breeding were not asked in the questionnaire. ? = the association of this study between the exposure and the outcome (purple line).*

Statistical results

Univariate results

Table 10 shows a summary of all numeric variables. Standard deviations were calculated after excluding the datapoints that were not applicable (NA). It shows that differences within one variable are enormous. PR for example, it ranges from 0 to a 100%. Number of cattle owned is another variable where a big difference between farms can be observed. Most farmers do not own many cows yet some own hundreds.

Table 4: Overview of numeric variables with their minimal (min) and maximal (max) values. The mean, median and standard deviation (SD) are given. For all variables the number of non-applicable (NA) data was given. Those datapoints were left out of all standard deviation calculations.

	Min	Max	Mean	Median	SD	NA
Distance (km)	1.00	33.00	12.62	10.00	8.11	0
Milk production	0.5	17.5	6.688	6.5	3.44	9
Margin/L	600.0	1400.0	892.2	750.0	213.78	9
Number of cattle owned	1.00	900.00	23.94	7.00	92.14	0
HDQ	0.0	488.0	159.8	140.5	100.22	0
Age	20.00	90.00	50.14	50.00	13.23	2
Pregnancy rate (%)	0.00	100.00	75.26	85.71	28.91	39

Numeric variables were checked for correlation. Margin of TZS per litre of milk has a clear negative correlation with the distance to Tanga city centre. A weak, but positive correlation is seen between HDQ and milk production (figure 11). Pregnancy rate was only calculated in 63/99 cases. Therefore, this variable may influence the rest of the correlation plot, as only 63 cases were included (figure 11). Leaving PR out of the correlation plot shows that a slightly stronger correlation is found between HDQ and milk production. A slightly stronger negative correlation was also found between margin and km (figure 12). The number of cattle owned and the margin per litre milk showed a weaker negative correlation when PR was excluded from the data plot.

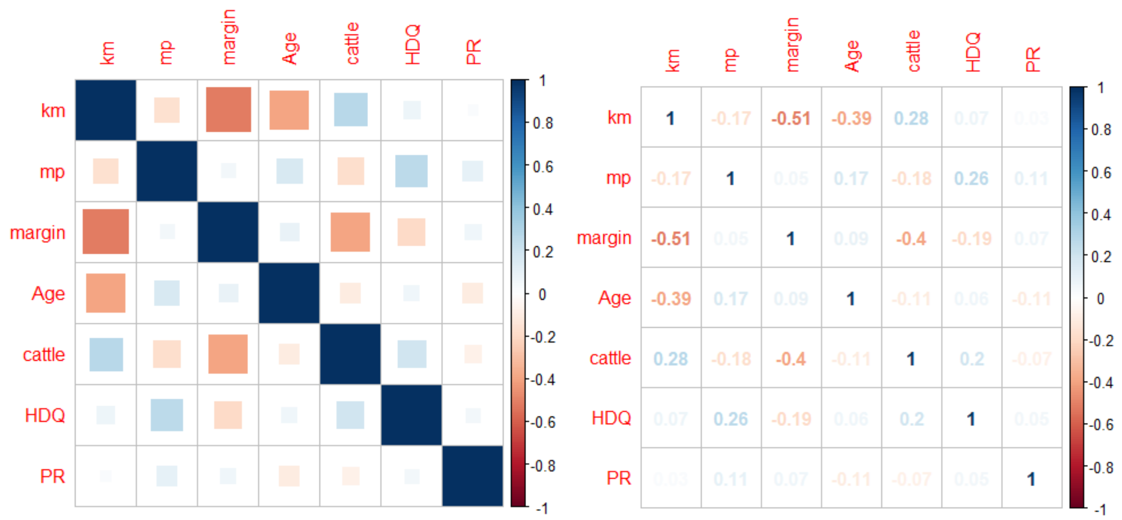

Figure 11: Spearman's correlation plot for different variables related to PR and one another. km= kilometre, mp= milk production, margin= Margin in TZs per litre milk, Age= age of the farmer, cattle = number of cattle owned, HDQ= heat detection quality, PR= pregnancy rate in %. The size and colour intensity of the squares display the degree of correlation. The numbers in the right plot give a more precise overview of the same data as in the left plot. Blue indicates a positive correlation; red indicates a negative correlation. The intensity of colours displays the strength of the correlation. Some squares and numbers are very light coloured, these have very weak correlation.

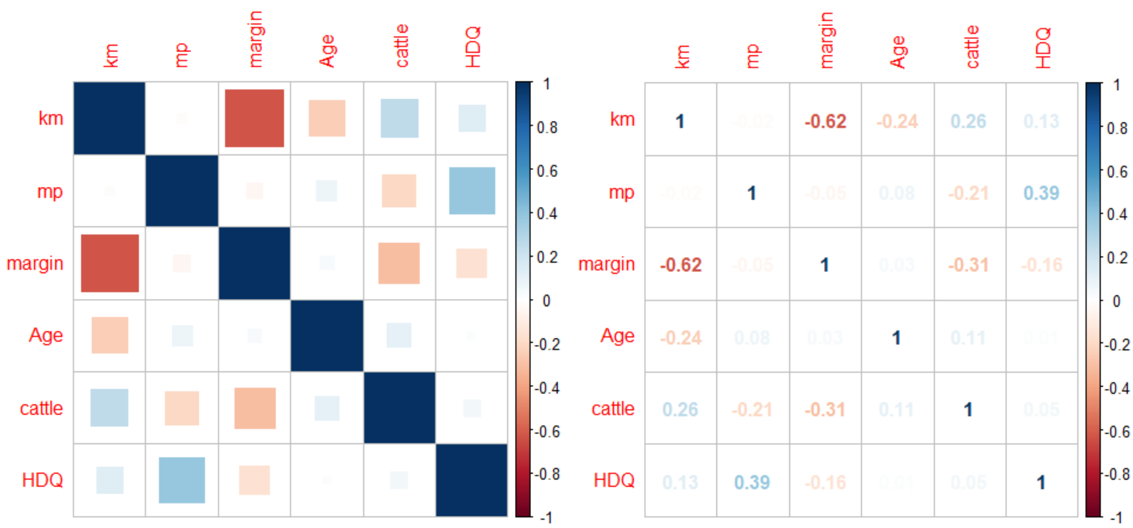

Figure 12: Correlation plot of variables without the pregnancy rate to display that the effect of the smaller data pool in the variable PR are minimal. Blue indicates a positive correlation; red indicates a negative correlation. The intensity of colours displays the strength of the correlation. Some squares and numbers are very light coloured, these have very weak correlation.

P-values for these numeric variables are presented in table 11. No significant variables in relation to the PR were found. Significance was found between km and margin. There is a negative relationship between the distance between the farm and Tanga city centre and the money earned per litre milk. The further away from the city centre the less money is paid for 1 litre of milk.

Table 5: p-values derived from Spearman's correlation. km= kilometre, mp= milk production, margin= Margin in TZS per litre milk, Age= age of the farmer, cattle = number of cattle owned, HDQ= heat detection quality, PR= pregnancy rate in %.

P

	km	mp	margin	Age	cattle	HDQ	PR
km		0.3467	0.0296	0.0821	0.1621	0.7474	0.9673
mp	0.3467		0.7033	0.5275	0.2766	0.5843	0.9446
margin	0.0296	0.7033		0.4979	0.0553	0.3139	0.8255
Age	0.0821	0.5275	0.4979		0.4157	0.9070	0.5143
cattle	0.1621	0.2766	0.0553	0.4157		0.5484	0.5696
HDQ	0.7474	0.5843	0.3139	0.9070	0.5484		0.7614
PR	0.9673	0.9446	0.8255	0.5143	0.5696	0.7614	

Categorical variables are presented in table 12. Farms using AI had the lowest mean PR of all breeding systems. Zero grazing also showed the lowest mean PR. Education level showed the lowest mean PR in the group of farmers that went to college, but numbers in these categories were small. No categories showed significance in relation to the PR.

Table 6: Overview of categorical variables in relation to the pregnancy rate

Variable	Number of data	Mean PR	SD	p-value
Type of breeding system				0.4026
AI	34	65.49	35.83	
DO	34	83.56	21.23	
Mix	9	83.3	19.25	
Non-AI	22	77.3	25.02	
Type of grazing system				0.4883
Mix	26	69.82	30.56	
Pastoral	49	81.08	23.82	
Restricted grazing	4	83.33	28.87	
Zero grazing	20	67.74	36.03	
Educational level				0.3418
No education	1	100.00	NA	
Primary	64	72.42	29.05	
Secondary	14	85.80	26.55	
Vocational	5	75.60	39.86	
College	8	66.67	47.14	
University	7	84.13	16.72	
Interval				0.5843
<1	34	76.61	22.41	
<4	7	58.33	52.04	
<8	13	78.17	24.04	
<12	20	88.33	22.75	
<20	12	67.00	36.94	

Based upon the AIC and p-value the variables breeding system, grazing system and educational level were found to be an improvement for the model if these variables were included in the multivariable model. All other variables did not show any improvement upon the AIC and were not significant. Therefore, all other variables were excluded from further testing.

Table 7: Variables and their AIC (Akaike information criterion) and p-value.

Variable	AIC improvement with variable included (yes/no)	p-value
Breeding system	Yes	0.019
Grazing system	Yes	0.031
Education level	Yes	0.015
Interval	No	0.184
Km	No	0.413
HDQ	No	0.650
Cattle	No	0.973
Mp	No	0.817
Margin	No	0.271
Age	No	0.374

Multivariate results

From table 14 it can be observed that all educational levels have a significant outcome and an OR with a 95% CI greater than one which implies a positive relationship with the pregnancy rate.
The intercept in this model is a combined intercept and consists of farmers within the AI group, a mixed grazing system and farmers who went to college. These variables seem to be significantly negatively associated with the pregnancy rate.
Breeding system shows an insignificant association with pregnancy rate for the drop out farmers and for the non-AI group. Both associations are significant. For grazing systems only the zero grazing system has a significant result but due to the fact that the 95% CI includes the value 1 an insignificant association was found between pregnancy rate and zero grazing.
Table 15 shows the number of non-applicable (NA) data. These could not be used in the multivariable model and were left out the dataset.

Table 8: Multivariable model: generalized linear mixed model. N= 45. Odds Ratio (OR), 95% Confidence interval (CI) lower (2.5%) and upper (97.5%) limit and p-value.

Variable	OR	2.5% Confidence interval limit	97.5% Confidence interval limit	p-value
Intercept (AI, college, mixed grazing)	0.2	0.09	0.5	0.0079
DO farms	1.0	1.0	2.4	0.0487
Mix farms	2.1	0.8	5.5	0.1110
Non-AI farms	1.6	0.9	2.8	0.0831
Mixed grazing				Ref
Pastoral grazing	0.8	0.5	1.3	0.4228
Restricted grazing	0.2	0.04	1.0	0.0518
Zero grazing	0.6	0.4	1.0	0.0488
No education				---
Primary education	5.8	2.1	16.1	0.0005
Secondary education	3.6	1.2	11.9	0.0264
Vocational	5.2	1.7	18.4	0.0021
College				Ref
University	2.0	2.0	19.1	0.0083

Table 9: Overview of non-applicable data per variable and their % in relation to the total number of data. Resulting in an usable dataset of 45 datapoints.

Variable	Number of NA	NA% of whole dataset
Distance (km)	0	0%
Milk production	9	9.1%
Margin/L	9	9.1%
Number of cattle owned	0	0%
HDQ	0	0%
Age	2	2.0%
Pregnancy rate (%)	39	39.4%
Breeding system	0	0%
Grazing system	0	0%
Education level	0	0%
Interval	13	13.1%

Discussion

The most important result from this study is that a negative association was found between the adoption of artificial insemination and the pregnancy rate. This is in line with findings from *Msangi et al. (2005)* who performed a larger study in the same area, results from their study show an association between using AI and a prolonged calving interval (36).

Mean pregnancy rate found in this study was 75%. This is high compared to other reports (37,38). Educational level and working experience have been the subject of some studies. Those imply that the higher the education level, the better the farmer will be at making new decisions and evaluating risks that come with those decisions (39). However, many years of working experience also is positively related to success. Although that relation appeared to be little less strong than the relation between education and success (40). This might explain why both lower and higher education level have a positive and significant relationship to the pregnancy rate, in this study the success level.

From this study no association between pregnancy rates and drop-out farmers could be observed. Although, it is known that due to poor AI service and many repetitions farmers in Tanga city council dropped out of the AI programme (41). Possibly leaving the disadvantages behind and with increased knowledge they started natural breeding.

Likewise, zero grazing had no strong statistical evidence of its association with the pregnancy rate. Even though there was no statistical evidence zero grazing might trouble the pregnancy rate. This might be due to a poorer display of oestrus for cows with limited space to move which is the case in zero grazing systems as was seen during this study (37). It has been described before that less oestrous behaviour was observed during the periods cows were tied after milking (25). Farms which use zero grazing in Tanga city council often tie their cattle as can be observed in figure 13.

Figure 13: Example of zero grazing in Tanga city council. Cows are housed in their own compartment and are often tied. The cow on the right shows a good example of this.

This study showed a slightly negative correlation between the distance from Tanga city centre in km and the margin of TZS per litre of milk. The further from the city the less money was earned per litre of milk. This is in accordance with findings of *Wassena et al. (2015)* (42).
Furthermore, it was seen that the mean number of cattle owned per farmer was 23. It was discussed by *van Vliet and van Eerdenburg (1996)* that in small herds (45-50 cows) heat detection might become more difficult as sometimes only one cow displayed signs of heat (21). In this study many very small herds were included. The HDQ score in this study showed plenty of room for improvement (mean =159.8, maximum achievable score = 571). This might show the difficulties with heat detection in small herds. However, pregnancy rates (mean= 75%) were not as low as might be expected with this information. Another study from *van Eerdenburg et al. (2002)* concluded that the small herds are not a reason for lack of (noticed) oestrous behaviour (20).

To the author’s knowledge, no other scoring system for the quality of heat detection existed prior to this study. This system, developed for this study, not only includes oestrous behaviour but multiple factors such as observation moment and duration. The factor ‘other work while watching the cows for heat’ was considered here as it influences quality of heat detection. A reduction of points was used to correct for this influence. The maximum achievable score was 571 points. The maximal achieved score recorded from the interviews was 488. However, the mean score was 159.8.

The twenty farmers looking for milk yield drop were not awarded with points for this observation as it is not a reliable sign of heat detection (35). It usually is present one day after oestrus.

Farmers, interviewed for this study, stated that an important reason for them to start AI was to be able to get a higher milk production. Mean milk production in the Tanga city council district is about 7 litres/day. Although the breed was not noted in this study, this finding is in accordance with findings of *Duguma et al. (2012),* who reported a mean milk production of 8 litres for crossbreeds. This implies that cows in Tanga city council do not produce badly as the population is a mixture of indigenous and crossbred cattle (43).

Improvements to this study
Improvements for the questionnaire
A true conception rate cannot be determined from a questionnaire. This would rather be the pregnancy rate, unfortunately, one question was not specific enough to calculate the pregnancy rate from this. However, it was decided that the pregnancy rate could be used after all as specificity of questions was lost during translation. Other examples of possible loss of details during translation of the interview are present within this study. Questions about the number of inseminations needed for pregnancy on average, how many cows were bred in the past year, how many of those got pregnant and how many inseminations were needed for those pregnancies were all in a similar category. The difference was in how the question was asked. However, a farmer answered that on average 75% of all cows bred in the last year, got pregnant from one insemination. To the question how many cows he tried to breed with the answer was ‘nine’. To the question how many of those cows got pregnant the answer was only two out of nine cows. This is only one example out of many that suggest that either details were lost in translation, the question was not clear, or farmers answered socially desirable. A combination of these factors is also possible.
From the start, clearer questions about the PR should have been included. Had that been done, more data would be available which would lead to stronger conclusions regarding the pregnancy rate. Besides the number of cows owned, also the breeds present should have been noted in categories such as zebu species, Holstein-Friesian and crossbred. For crossbred’s information on the type of crossbred could have been collected. As it was known that not only Holstein-Friesian breeds were used in AI but also Jersey, Ayshire and Kiwicross were (sometimes) available.
Chawala et al. 2020 looked at the breeds present on each farm and found that pure bred exotic breeds, such as Holstein-Friesian, produce less milk in sub-Saharan Africa then the same breeds do in other parts of the world (44). This is interesting as farmers spoke of a huge supposedly impact of these pure and crossbreeds. However, these might be lower for exotic breeds in Sub-Saharan Africa. If information on breeds present on farms were recorded an estimation could be made for the milk production per breed in the Tanga region. This could help farmers decide whether AI would meet their expectations with regards to milk production.

Improvements for method of HDQ calculation.

Farmers were asked about oestrous behaviour signs they used for the detection of heat. These were scored with points available through the system developed by *Van Eerdenburg et al. (1996).* Although, not all answers given by the farmers were available in this scoring system. Therefore, their other answers were evaluated and if useful included in the scoring system as presented in this study.

Improvements for data notation and analysis

On forehand, more thought should have put in the notation systems and categories. Clearer categories should have been present at the beginning of this study.

At the start of data analysis farm factors were rounded in order to work properly in R.

During analysis it became clear that the number of data in the model and the variables considered in the multivariable model changed outcomes. For example, including educational level in the model resulted in a significant intercept and a significance for the non-AI farmers. As opposed to a model where only breeding and grazing system were included. A reduction of data used in the model as a result of inclusion of all variables thought to be relevant and excluding all NA data points, the AIC of variables changed. For example, in a model with 53 datapoints km and mp should have run in the model instead of education. However, with only 45 datapoints available to run in the model these variables were no longer considered to be an improvement to the model. Therefore, it should be noted that if this study was repeated with more data it might not show the same results.

While composing the DAG diagram it was believed that the interval between heat detection and breeding was a result of the breeding system. However, it is debatable that because of long waiting periods in which the farmer waits for the AI technician to arrive that this might influence farmers' choice to start, continue or end the AI programme. Even though this is a debatable and possible crucial finding -as the variable interval threw out a substantial amount of data because of many NA datapoints- one of the definitions that define a confounding variable that it cannot be a result of the exposure (breeding system) (45). This is as stated with the 'old' concept of confounding variables. A DAG diagram only shows if confounding is present at all. This often involves complex diagrams (46). Therefore, variables of importance might have been missed and left out of the DAG diagram despite intensive labour.

Recommendations for further research

Future research can further develop and assess the heat detection quality scoring system. Part of the system involves an oestrous behaviour score, as factors were added these should also be assessed. The system as a total should also be assessed, so that farmers can be advised based on this score and the component that is responsible for the high or low score. This would be beneficial to the farmers as they receive a more detailed and specific advice. With these specific advices economic growth might be established.

Other parts that need to be included in these types of studies are other factors that influence pregnancy rates. Factors such as quality and quantity of food, health status, body condition score, heat stress, water quality and quantity should be included in future research.

Aspects such as transportation of frozen sperm and the access to liquid nitrogen might also give valuable insight on how to improve AI in Tanga city council.

Recommendations for farmers in Tanga city council and AI technicians.
A farmer told us that sometimes no AI technician shows up. Resulting in a missed opportunity for fertilization. It would be beneficial for the farmer if AI technicians let the farmer know if any problems or delays have appeared. In that way the farmer can try if another AI technician is available or a bull can be arranged. This way opportunities for fertilization are still used even though the AI technician of choice could not make it in time. If farmers are satisfied about communication and AI in general, they are more likely to use it in the future.
Furthermore, it was found that the mean milk production of cattle in Tanga city council is similar to that of crossbred cattle in other studies. Therefore, it should be explained clearly that an increase in milk production might be expected. However, a realistic improvement should be explained as well as other factors that influence milk production.
Last, the heat detection quality scoring system was developed. This might be used by AI-technicians and farmers as to evaluate 'room for improvements' in their heat detection system. Instead of 'just' evaluating whether AI application was successful or not.

Conclusion
Adoption of AI along with mixed grazing system and a college education level were significantly negatively associated with the pregnancy rate. Furthermore, farmers in the drop out group showed a positive and significant association with pregnancy rate.
This implies that adoption of AI not necessarily is the best system in order to achieve a high pregnancy rate. However, farmers of all breeding systems showed PR of 100%. Although, the AI group showed the lowest PR results.
Zero grazing showed a negative association with PR. The variable highest educational level was highly significant on all levels. Other research showed that not only education level but also years of experience are important for a successful business.
During this study a scoring system for heat detection quality was developed. This system along with the results of this study might help AI technicians and farmers in the Tanga city council district to gain more insight in their HDQ.
However, it is important to understand that the data records, that could be used for a multivariable model, were limited. Therefore, it is possible that upon repetition of this study other conclusions could be drawn.

Acknowledgements
I would like to thank Dr. Lyimo and Dr. Mbata for hosting this research opportunity and showing us around in Tanga. Thank you to Dr. Frank van Eerdenburg who supervised with great help.
Furthermore, I would like to thank livestock field officers Mr. Chonga and Mr. Mtera for their help arranging transportations, directions and translations. Without their help this would not been possible.
A huge appreciation goes out to all farmers who made time for us to do the interview. Once again without their cooperation this study would not exist.
Furthermore, I would like to thank Matthijs Kemp who helped with R troubleshooting.
Last, I would like to thank Marianne de Jong without whom this journey and study would not have been as much fun as it was.

References

1. P.L.Senger. The follicular phase. In: Pathways to pregnancy and parturition. 3rd ed. Current Conceptions Inc.; 2012. p. 160–79.
2. P.L.Senger. Reproductive cyclicity - Therminology & Basic concepts. In: Pathways to pregnancy and parturition. 3rd ed. Current Conceptions Inc.; 2012. p. 140–59.
3. Roelofs J, López-Gatius F, Hunter RHF, van Eerdenburg FJCM, Hanzen C. When is a cow in estrus? Clinical and practical aspects. Vol. 74, Theriogenology. 2010. p. 327–44.
4. Arthur GH. Endogenous and exogenous control of ovarian cyclicity. In: Noakes, David E. Parkinson, Timothy J. England, Gary C.W. Arthur GH, editor. Arthur's Veterinary Reproduction and Obstetrics. 8th ed. W.B. Saunders Elsevier; 2001. p. 3–53.
5. Roelofs JB, Van Eerdenburg FJCM, Soede NM, Kemp B. Various behavioral signs of estrous and their relationship with time of ovulation in dairy cattle. Theriogenology. 2005 Mar 15;63(5):1366–77.
6. Hawk HW. Transport and Fate of Spermatozoa After Insemination of Cattle. J Dairy Sci. 1987;70(7):1487–503.
7. Hunter R, Greve T. Could Artificial Insemination of Cattle be More Fruitful? Penalties Associated with Ageing Eggs. Reprod Domest Anim [Internet]. 1997 May 1 [cited 2020 Aug 2];32(3):137–41. Available from: http://doi.wiley.com/10.1111/j.1439-0531.1997.tb01271.x
8. Gordon I. Laboratory production of cattle embryos [Internet]. Second edi. Laboratory production of cattle embryos. Wallingford: CABI publishers; 2003 [cited 2020 Jul 31]. 178–180 p. Available from: https://books.google.nl/books?hl=nl&lr=&id=QO-KlTSjqCUC&oi=fnd&pg=IA1&dq=Gordon+2003+laboratory+production+embryos&ots=v-3L9kJpis&sig=tPIowZzRBta5HpXqngrc_qdHAnw#v=onepage&q=Gordon 2003 laboratory production embryos&f=false
9. Williams WF, Yver DR, Gross TS. Comparison of Estrus Detection Techniques in Dairy Heifers. J Dairy Sci. 1981;64(8):1738–41.
10. Maatje K, Loeffler SH, Engel B. Predicting Optimal Time of Insemination in Cows that Show Visual Signs of Estrus by Estimating Onset of Estrus with Pedometers. J Dairy Sci. 1997;80(6):1098–105.
11. Rajamahendran R, Robinson J, Desbottes S, Walton JS. Temporal relationships among estrus, body temperature, milk yield, progesterone and luteinizing hormone levels, and ovulation in dairy cows. Theriogenology. 1989 Jun 1;31(6):1173–82.
12. Rorie RW, Bilby TR, Lester TD. Application of electronic estrus detection technologies to reproductive management of cattle. Theriogenology. 2002 Jan 1;57(1):137–48.
13. Lane AJP, Wathes DC. An Electronic Nose to Detect Changes in Perineal Odors Associated with Estrus in the Cow. J Dairy Sci. 1998;81(8):2145–50.
14. Canfield RW, Butler WR. Accuracy of predicting the LH surge and optimal insemination time in Holstein heifers using a vaginal resistance probe. Theriogenology. 1989 Apr 1;31(4):835–42.
15. Schneider HJ, Castleberry RS, Griffin JL. COMMERCIAL ASPECTS OF BOVINE EMBRYO TRANSFER. 1980.
16. Roelofs JB. Prediction of ovulation and optimal insemination interval [Internet]. 2008 [cited 2020 Jun 25]. Available from: https://www.researchgate.net/publication/40496104

17. Wright JM. THERIOGENOLOGY NON-SURGICAL EMBRYO TRANSFER IN CATTLE EMBRYO-RECIPIENT INTERACTIONS. 1981.

18. Roelofs JB, Graat EAM, Mullaart E, Soede NM, Voskamp-Harkema W, Kemp B. Effects of insemination-ovulation interval on fertilization rates and embryo characteristics in dairy cattle. Theriogenology. 2006 Dec 1;66(9):2173–81.

19. Dalton JC, Nadir S, Bame JH, Noftsinger M, Nebel RL, Saacke RG. Effect of time of insemination on number of accessory sperm, fertilization rate, and embryo quality in nonlactating dairy cattle. J Dairy Sci. 2001 Nov 1;84(11):2413–8.

20. Van Eerdenburg FJCM, Karthaus D, Taverne MAM, Merics I, Szenci O. The relationship between estrous behavioral score and time of ovulation in dairy cattle. J Dairy Sci. 2002 May 1;85(5):1150–6.

21. Van Vliet JH, Van Eerdenburg FJCM. Sexual activities and oestrus detection in lactating Holstein cows. Appl Anim Behav Sci. 1996 Oct 1;50(1):57–69.

22. Lyimo ZC, Nielen M, Ouweltjes W, Kruip TAM, Van Eerdenburg FJCM. Relationship among estradiol, cortisol and intensity of estrous behavior in dairy cattle. Theriogenology. 2000 Jun;53(9):1783–95.

24. LeBlanc SJ, Leslie KE, Ceelen HJ, Kelton DF, Keefe GP. Measures of Estrus Detection and Pregnancy in Dairy Cows after Administration of Gonadotropin-Releasing Hormone Within an Estrus Synchronization Program Based on Prostaglandin F2α. J Dairy Sci. 1998 Feb 1;81(2):375–81.

25. Van Eerdenburg FJCM, Loeffler HSH, van Vliet JH. Detection of oestrus in dairy cows: A new approach to an old problem. Vet Q [Internet]. 1996 [cited 2020 Jul 31];18(2):52–4. Available from: https://www.tandfonline.com/action/journalInformation?journalCode=tveq20

26. Woldu T, Giorgis YT, Haile A. Factors affecting conception rate in artificially inseminated cattle under farmers condition in Ethiopia. J Cell Anim Biol [Internet]. 2011 [cited 2020 Jul 4];5(16):334–8. Available from: http://www.academicjournals.org/JCAB

27. Mukasa-Mugerwa E, Tegegne A, Teklu Y. Characterization of service intervals and frequency of short oestrus cycles in Zebu (Bos indicus) cows in Ethiopia. 1991.

28. Dawuda PM, Eduvie LO, Esievo KAN, Molokwu ECI. Silent Oestrus Manifestation in Nigerian Bunaji Zebu Cows. 1989.

29. Rao TKS KNKPCS and PN. Heat detection techniques in cattle and buffalo. Vet.world [Internet]. 2013 [cited 2020 Jul 3];6(6):363–9. Available from: www.veterinaryworld.org

30. Heres L, Dieleman SJ, van Eerdenburg FJCM. Validation of a new method of visual oestrus detection on the farm. Vet Q [Internet]. 2000 [cited 2020 Jul 3];22(1):50–5. Available from: https://www.tandfonline.com/doi/abs/10.1080/01652176.2000.9695023

31. Mcgowan JE, Burke CR, Jago JG. Validation of a technology for objectively measuring behaviour in dairy cows and its application for oestrous detection.

32. Inchaisri C, Jorritsma R, Vos PLAM, van der Weijden GC, Hogeveen H. Economic consequences of reproductive performance in dairy cattle. Theriogenology. 2010 Sep 15;74(5):835–46.

33. Wageindicator [Internet]. [cited 2020 Aug 28]. Available from: https://wageindicator.org/salary/minimum-wage/tanzania/6185-mainland-agricultural-

services

34. Dransfield MBG, Nebel RL, Pearson RE, Warnick LD. Timing of Insemination for Dairy Cows Identified in Estrus by a Radiotelemetric Estrus Detection System. J Dairy Sci. 1998;81(7):1874–82.

35. Diskin MG, Sreenan JM. Expression and detection of oestrus in cattle. Vol. 40, Reprod. Nutr. Dev. 2000.

36. Msangi BSJ, Bryant MJ, Thorne PJ. Some factors affecting reproductive success in crossbred dairy cows on smallholder farms in coastal north-east Tanzania [Internet]. Vol. 37, Tropical Animal Health and Production. Springer; 2005 [cited 2020 Sep 2]. p. 413–26. Available from: https://link.springer.com/article/10.1007/s11250-005-6855-6

37. Landivar C, Galina CS, Duchateau A, Navarro-Fierro R. Fertility trial in Zebu cattle after a natural or controlled estrus with prostaglandin F2 Alpha, comparing natural mating with artificial insemination. Theriogenology. 1985;23(3):421–9.

38. Pursley JR, Wiltbank MC, Stevenson JS, Ottobre JS, Garverick HA, Anderson LL. Pregnancy Rates Per Artificial Insemination for Cows and Heifers Inseminated at a Synchronized Ovulation or Synchronized Estrus. J Dairy Sci. 1997 Feb 1;80(2):295–300.

39. D M G Njarui, J M Kabirizi, J K Itabari, M Gatheru AN and SM. Production characteristics and gender roles in dairy farming in peri-urban areas of Eastern and Central Africa. Livest Res Rural Dev [Internet]. 2012 [cited 2020 Aug 27];24(7). Available from: https://www.lrrd.cipav.org.co/lrrd24/7/njar24122.htm

40. Robinson PB, Sexton EA. The effect of education and experience on self-employment success. J Bus Ventur. 1994 Mar 1;9(2):141–56.

41. Jong MJK de. A One Health approach towards artificial insemination in cattle in Tanga, Tanzania. 2019 [cited 2020 Jun 25]; Available from: http://dspace.library.uu.nl/handle/1874/382998

42. Wassena F J, Mangesho W E, Chawala A, Laswai G H, Bwire J M N, Kimambo A E, Lukuyu B SG and MBL. Effects of season and location on cattle milk produced and producer milk prices in selected villages of Tanga and Morogoro Regions, Tanzania. Livest Res Rural Dev [Internet]. 2015 [cited 2020 Aug 13];Volume 27,. Available from: http://www.lrrd.cipav.org.co/lrrd27/10/wass27191.html

43. Duguma Eticha B, Kechero Y. Productive and reproductive performance of Zebu X Holstein-Friesian crossbred dairy cows in Jimma town, Oromia, Ethiopia The assessment of water resources quality View project [Internet]. [cited 2020 Jun 25]. Available from: https://www.researchgate.net/publication/234026629

44. Chawala AR, Mwai AO, Peters A, Banos G, Chagunda MGG. Towards a better understanding of breeding objectives and production performance of dairy cattle in sub-Saharan Africa: a systematic review and meta-analysis. 2020 [cited 2020 Aug 3]; Available from: http://www.cabi.org/cabreviewshttp://www.cabi.org/cabreviews

45. Jager KJ, Zoccali C, MacLeod A, Dekker FW. Confounding: What it is and how to deal with it. Kidney Int. 2008 Feb 1;73(3):256–60.

46. Suttorp MM, Siegerink B, Jager KJ, Zoccali C, Dekker FW. NDT Perspectives Graphical presentation of confounding in directed acyclic graphs. Nephrol Dial Transpl [Internet]. 2015 [cited 2020 Aug 11];30:1418–23. Available from: https://academic.oup.com/ndt/article/30/9/1418/2459917

Appendix

Appendix A

Farmers questionnaire

	Name:	Swahili/English
1	District	
2	Village	
3	Male/female	
4	Age	
5	Highest level of education	
	Primary	
	Secondary	
	Vocational	
	College	
	University	
6	How long have you been a farmer?	
7	Do you have any other job besides being a farmer?	
	If yes, How much time do you spent doing off-farm work?	
8	Number of children?	
9	If you are unable to continue farming is there someone who will take care of your farm?	
10	Type of farm	
	Zero grazing and free walking	
	Zero grazing and tied up	
	Restricted grazing	
	Pastoral	
	Mixed and tied up	
	Mixed and free walking	
11	Distance to city	
12	Milk yield/cow/day	
13	Margin L milk	
14	Do you have access to information on AI?	
	If yes, where?	
15	How many cows do you own?	
16	How many cows do you own that are being milked?	
17	Do you adopt AI on your cows?	
18	**Why did you start with AI?**	
19	***If you don't adopt AI, why not?***	
20	**How many cows do you adopt AI on?**	
21	**How much do you pay for insemination?**	
22	How many times/day do you watch cows for heat?	
23	On part of the day do you watch for heat?	
24	How long do you watch each time?	
25	Do you do other work while watching cows for oestrus?	
26	What are the signs of heat you pay attention to?	
	Being mounted while standing	1
	Mounting of other cows	2
	Flehmen	3
	Increase in activity	4
	Chin resting	5
	Sniffing other cows	6
	Mounting while not standing still	7
	Body temperature	8

	Colour of vulva mucosa	9
	Mucus secretion of vulva	10
	Decreased feed uptake	11
	More alert (raising ears)	12
	More noises	13
	Reaction upon touching genitals	14
	Enlargement of vulva	15
	Other	16
27	**How long after you first detect heat, you call the AI technician?**	
	If you don't adopt AI: How long after detection the bull and cow mate?	
28	**How long after you called the AI tech insemination takes place?**	
29	Do you measure hormone concentrations?	
	If yes, which one?	
30	Did you have/follow training in heat detection?	
	Yes, from a veterinarian	
	Yes, from AI technician	
	Yes, from other farmer(s)	
	No	
31	**Is there a choice in inseminators?**	
	Yes I choose	
	No there is only one	
	No company/project chooses	
32	**Who is your main AI tech?**	
33	**How long have you been using AI instead of a bull?**	
34	**Can you choose which bull delivers the sperm for AI?**	
35	(If you could choose)What are the characteristics for bull selection?	
	Exterior	
	Milk yield	
	Fertility	
	Muscle gain	
	Resistance to climate	
	Higher milk fat %	
	Other	
	Healthy bull (non genetic factor)	
36	**Do you still own a bull?**	
37	**If yes, do you use this bull for natural breeding?**	
38	**Does someone manually check the cow if she is in oestrus before insemination?**	
	Yes, veterinarian	
	Yes, inseminator	
	Yes, farmer	
	No	
39	What is the average amount of inseminations per cow before it gets pregnant?/ number of services from bull	
40	What is the conception rate? (from system)	
41	How many cows get pregnant from their first insemination in the last year?/ number of services from bull	
42	How many cows are pregnant right now.	
43	How many cows did you breed with, in the past year, and how many of those got pregnant?	
44	Possible benefits of AI	
	Healthier farmer and family	
	Increased milk yield in offspring	
	Increased fertility in offspring	
	Less infectious diseases transmitted	

	Increased fertility in cows to be inseminated due to lower risk of venereal diseases	
	Healthier calves	
	Better record keeping	
	Increased value of offspring	
	Genetic progress in general	
	Possibility of crossbreeding with international breeds	
	Saves time compared to bringing cow to bull	
	Other	
45	Most important advantage	
46	Possible disadvantages of AI	
	Costs of insemination	
	Time before technician arrives after calling	
	Decreased value of bulls	
	Farmer has too little knowledge of AI to check technician	
	Loss of local genetic traits	
	Need for closer care of offspring because of higher value	
	Other	
47	Most important disadvantage	
48	Do you want to farm sustainably?/without harming environment	
49	Do you discuss AI with other farmers?	
	If yes, how many times/year	
	How do you discuss? (meetings, whatsapp, informal)	
50	**How do you fixate the animal that is presented for AI?**	
51	**Email:**	

Circle the applicable: AI, *Non-AI*, Both

Appendix B

Overview of farmers interviewed per village.

Pingoni is greatly represented as 26 farmers lived there were interviewed.

Village	Number of farmers interviewed (n=101)
Chunda kota	1
Donge	5
Duga	1
Kange	1
Kasera	1
Kichangani	6
Kiomoni	1
Kivumbiti tifu	1
Kombezi b	1
Kwenjega b	1
Machui	5
Machui kuu	1
Maere	1
Mafuriko	7
Magoani b	1
Magaoni b mabawa	1
Mapinduzi	1
Masiwani	2
Maweni	2
Mbolea	1
Mbolea mzaingoni	1
Mikanjuni a	2
Mleni	6
Mzingani/pingoni	1
Neema	3
Nguvumali	1
Nguvumali a	1
Pande b	5
Pingoni	24
Pingoni/ town	1
Pongwe	3
Pongwe /pangani	1
Sahare	1
Swahili	1
Tangasisi	4
Tongoni	1
Usagara	1
Usagara Kijijni	1

Appendix C

Number of farmers and the moments they check their cows for heat.

Part of the day farmer checks for heat	Number of answers (n=99)
Morning	4
Afternoon	0
Evening	3
Morning and night	1
Morning and evening	37
Morning and afternoon	8
Morning, afternoon and evening	2
Morning, noon and evening	4
Morning, afternoon and evening	9
Morning, noon and night	1
Morning, afternoon, evening (and night)	1
Morning, evening and night	3
Morning, noon, afternoon and late evening plus an extra time in between	2
Morning, afternoon and evening plus an extra time in between	1
All day long	3
All day long, but watched by herdsman	3
Random	1
Only when the farmer hears more noises than normal	1
Never	4
Unknown	1
Non-applicable (NA) data	7

Appendix D

Three-way table of the pregnancy rate. It is displayed per grazing system and per breeding system

	AI	DO	Mix	Non-ai	
Mix					
0-20% PR	2	0	0	0	
20-40% PR	1	0	0	0	
40-60% PR	2	0	0	0	
60-80% PR	0	2	1	0	
80-100% PR	2	3	1	1	
Pastoral					
0-20% PR	0	0	0	0	
20-40% PR	0	1	0	1	
40-60% PR	1	1	0	2	
60-80% PR	1	3	0	2	
80-100% PR	2	8	1	5	
Restricted grazing					
0-20% PR	0	0	0	0	
20-40% PR	0	0	0	0	
40-60% PR	0	0	0	1	
60-80% PR	0	0	0	0	
80-100% PR	1	0	0	1	
Zero grazing					
0-20% PR	0	0	0	0	
20-40% PR	1	0	0	0	
40-60% PR	1	1	0	0	
60-80% PR	2	0	1	0	
80-100% PR	5	1	0	0	