Comparative Study between Fixed-Time Artificial Insemination and Natural Mating on Reproductive Performance (conception and pregnancy rates) of Mpwapwa breed cows in Tanzania

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Abstract

The aim of this project was to assess whether using a $PGF_{2\alpha}$ synchronization protocol in Mpwapwa cattle would improve reproductive performance. A standard 14-day $PGF_{2\alpha}$ synchronization protocol with a single FTAI was compared to NM over a 12-weeks breeding season. At the end of the study, 39/100 cows were pregnant in the FTAI group and 49/100 cows were pregnant in the NM group. This difference was not statistically significant (P=0.21), although the odds ratio of pregnancy was lower in the FTAI group than the NM group (unadjusted RR=0.8; 95% - CI 0.58-1.09). However, cattle in the $PGF_{2\alpha}$-treated group were only inseminated once, whereas the NM group could be naturally mated on multiple occasions during the breeding season; In addition, the use of $PGF_{2\alpha}$ allowed the use of AI, which is not feasible under most Tanzanian systems when cattle come into oestrus naturally. Thus, the results of this study suggest that $PGF_{2\alpha}$ -based synchronization and FTAI, particularly if used alongside natural mating, can improve the reproductive performance of Mpwapwa breed cattle as well as allowing for greater genetic gain than occurs with naturally mated cattle.

The proportion of cows that came into heat and displayed behavioural signs after administration of the first $PGF_{2\alpha}$ injection was very low (only 10/100 cattle). The reason for this poor response is unclear. It could be that oestrus detection was not very effective, or that a higher than expected proportion of cattle did not have a responsive CL. The most likely cause of the latter is a higher proportion of cattle in anoestrus. Further investigation of the reproductive state of Mpwapwa cattle at the start of the breeding season would identify how important anoestrus is as a cause of poor reproductive performance. If anoestrus is common, identifying cattle in anoestrus at the start of the season could be useful, as they could be treated using progesterone-based programmes and cattle with a CL could be treated with $PGF_{2\alpha}$.

Key words: Mpwapwa breed cattle, $PGF_{2\alpha}$ synchronization protocol, FTAI, NM, Reproductive performance.

Declaration

Dedication

My dedication concerning this thesis goes firstly to my beloved wife Anna Abraham Dumly for her sacrifice and words of motivation and inspiration meant a lot to the completion of this work, my sons Mruga Kabuni Thomas and Imori Kabuni Thomas for their tolerance of missing my presence at home while l was away on studies. Secondly to my mother Mesuri Abunuasi Mtoka and my late father Thomas Kabuni for building a good foundation in my education life. Thirdly to my brothers George Thomas Kabuni, Mtoka Thomas Kabuni, Pascas Mragili and my sisters Muse Thomas Kabuni and Mugesi Thomas Kabuni for their support. Lastly to my supervisors Professor Tim J. Parkinson and Doctor Richard Laven for their guidance and assistance in making sure this thesis is successfully completed.

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List of abbreviations

AI Artificial Insemination

AART Assisted Animals' Reproductive Technologies

BCS Body Condition Score

BSE Breeding Soundness Evaluation/Examination

CI Confidence Interval

CL Corpus Luteum

CIDR Controlled Internal Drug Release (Intravaginal progesterone insert)

ET Embryo Transfer

FTAI Fixed-Time Artificial Insemination

FSH Follicle Stimulating Hormone

GnRH Gonadotrophin Releasing Hormone

LH Luteinizing Hormone

LU Livestock Unit

MOET Multiple Ovulation and Embryo Transfer

NM Natural Mating

PD Pregnancy Diagnosis

$PGF_{2}\alpha$ (PGF/PG/ $F_{2\alpha}$) Prostaglandin hormone

RR Relative Risk

SC Scrotal Circumference

Chapter 1

General introduction

The total land size of Tanzania is 95.5 million hectares (Ha), of which 44 million Ha have been classified as suitable land to be used for agricultural activities (MLFD, 2010). However, 10.6 million Ha of agricultural land are utilized for crops production, whereas about 50 million Ha of rangelands are suitable for livestock keeping. Currently, only 24 million Ha of total rangelands are being used to support 12.1 million ruminants (MLFD, 2010). The remaining unused rangelands are prone to tsetse flies making them unfit for livestock farming (MLFD, 2010). Table 1 summarises the land uses for agricultural activities in Tanzania.

Table 1 Land resource and livestock population in Tanzania

Land resource and Livestock population in Tanzania		
Type of Ruminant Livestock	Million ha	
Total land area	95.5	
Arable land (ha)	44.0	
Cultivated land	10.56*	
Area suitable for irrigation	29.4	
Area under irrigation	0.29	
Area under medium and large scale farming	1.5	
Rangeland	50.0	
Land under livestock	24.0	
Per capital land holding (ha/head)	0.1	
Livestock population		
	Millions	Million Livestock Units
Cattle	19.2	9.6

Goats	13.7	1.37
Sheep	3.6	0.36
Pigs	1.9	0.19
Poultry (indigenous +exotic)	58.0	0.58
Total	96.4	12.1

Source: (MLFD, 2010). *LU Conversion factor: cattle and Buffaloes, 0.5; Sheep and goats 0.1 and poultry 0.01 (FAO, 2003) * 24% of arable land is currently cultivated (2008)*

Livestock keeping in Tanzania is among the major agricultural activities practiced by pastoralists and agro-pastoralists in different agro-ecological zones found in the country (MLFD, 2010). Livestock keeping has been reported to contribute towards attaining development goals of the National Growth and Reduction of Poverty (MLFD, 2010). The livestock industry contributes 13% to the Agricultural Gross Domestic Product in Tanzania, which in turn contributes 3.8% to the National Gross Domestic Product in 2010 (MLFD, 2010). This contribution showed a decreasing rate as compared to the performance of livestock industry and its contribution to National Gross Domestic Product in 2009 which was 4.0 % (MLFD, 2010). The declined performance of the livestock industry was as a result of low reproductive rates, low growth rates, high mortality rates, and poor quality final products produced by the industry (MLFD, 2010).

Nevertheless, both meat and milk production increased from the year 2000/2001 to 2009/2010; milk production increased from 814 million to 1.64 billion litres while meat production increased from 323,000 to 449,673 tones (MLFD, 2010). Over the same period of time per capita consumption of meat increased from 5.3 to 5.9kg, while milk consumption increased from 22 to 24 litres (MLFD, 2010). However, these consumption levels are well below the FAO recommendations of 50kg of meat and 200L of milk per capita (MLFD, 2010). According to the Tanzanian Ministry of Livestock and Fisheries Development (MLFD), 70% of annual milk production of the Tanzania dairy industry originates from the traditional sector (i.e. from indigenous cows), whereas the remaining 30% milk production comes from the commercial sector (i.e. from single-purpose dairy cows) (MLFD, 2010). In contrast, 53% of annual meat production originates from indigenous beef cattle breeds (such as the Tanzania Short Horn Zebu (TSZ), Boran, Ankole and Mpwapwa breeds: (MLFD, 2010), while the remaining 47% of the meat production comes from poultry, pigs, sheep and goats (MLFD, 2010). The National Ranching Company (NARCO) is the dominant producer of beef production commercially within the country (MLFD, 2012), although there are many other individual commercial beef-farming enterprises. However, the current level of productions in the traditional sector and of NARCO is reported to be below target (MLFD, 2010). Tables 2 to 4 summarise the figures for livestock production in Tanzania over recent years.

Table 2 Production and Consumption of Livestock and Poultry Products from 2005-2009

	Unit	2005	2006	2007	2008	2009	% Change over 2008	Average % annual increase
Human population in million	Persons	36.2	37.5	38.3	39.3	40	1.8	2.5
Product								
Meat								
Beef	Tons	210,370	180,629	218,976	225,178	243,943	8.3	4.6
Lamb	Tons	78,579	80,936	81,173	82,884	86,634	4.5	2.5
Pork	Tons	29,925	31,721	33,307	36,000	38,180	6.1	6.3
Chicken	Tons	69,420	77,280	77,250	78,168	80,916	3.5	4.0
Total	Tons	388,294	370,566	410,706	422,230	449,673	6.5	5.3
Per capita consumption	Kgs per year	11	10	11	11	12	9.1	
Milk								

Traditional cattle	Litres (000)	920,000	941,815	945,524	980,000	997,261	1.8	2.0
Dairy Cattle	Litres (000)	466,400	470,971	475,681	520,000	652,596	25.5	9.2
Total		1,386,400	1,412,786	1,421,205	1,500,000	1,649,857	10.0	4.5
Per capita consumption	Litres per year	38	40	41	42	43	2.4	
Chicken eggs	Number (000)	2,145,000	2,230,900	2,690,000	2,806,350	2,917,875	4.0	8.2
Per capita consumption		53	64	64	72	75	4.2	9.4

Source: (MLFD, 2010)

Table 3 Dairy Cattle Productivity in the Traditional and Improved Dairy Herd

Parameter	Traditional Sector	Improved dairy cattle (Kitulo)	Small holder dairy (Tanga/Kagera)	Ideal standard
Calving rate (%)	30-50	55-73	40-50	80
Calving interval (months)	18-24	15-21	17-18	12
Age at first calving (months)	36-48	30-36	43-46	27-30
Pre-weaning mortality (%)	25-40	4.3	5-6	<5.0
Calf mortality (%)	>25	3.3	5-6	<10.0
Adult mortality (%)	8-10	1.3	<1.0	<5.0
Mature weight	200-300	250-300		300-500
Lactation yield	160-250	2800-3500	1500-2000	2500-3500
Lactation length	200	300	270-300	305

Source: (MLFD, 2010) *adapted from Smallholder Dairy Support Programme (2005)*

Table 4 Beef Cattle Productivity in the Traditional Sector and in the Commercial NARCO Ranches

Parameter	Traditional Sector	Commercial NARCO Ranches	Expected standard
Area (ha)	23,376,200	609,164	-
Number of stock (2005) (million)	18.2	0.1	-
Av. stocking rate (acres/LU)	<3	4-5	4-5
Calving rate	40-50	55-73	80
Calving interval (months)	18-24	15-21	12
Pre-weaning mortality (%)	25-40	4.3	<5.0
Calf mortality (%)	>25	3.3	<10
Adult mortality (%)	8-10	1.3	<5.
Mature weight	200-300	250-300	350-400
Annual off take rate (%)	8-10	12	15
Carcass weight (kg)	100-175	120-175	200-250
Age at slaughter (years)	6-7	2-3	2-3

Source: (MLFD, 2010)

Currently the use of reproductive technologies such as FTAI and ET in the Tanzanian beef and dairy cattle industry is below target (MLD, 2006; MLFD, 2010). Despite the availability of biotechnology techniques such as germplasm conservation, MOET and genetic transformation along with strategies to stimulate their uptake (MLD, 2006), application and utilisation has not been at a level to be effective in improving the national herd (MLFD, 2010). AI had been widely used by the Tanzanian dairy industry; this has resulted in positive progress due to better dairy cow performance, particularly in terms of reproduction and productivity (MLFD, 2010). Several projects and programmes have been developed across Tanzania to stimulate the use of AI, in order to spread the gains seen on dairy farms across the cattle industry. However, in beef cattle, they have had a very limited impact (MLFD, 2010). Lack of an adequate AI infrastructure and insufficient trained AI technicians, remain key issues preventing uptake, alongside poor farmer training, inherently poor fertility of cattle and the absence of an effective national breeding programme (MLD, 2006; MLFD, 2010).

In Tanzania, almost all beef cattle are farmed under pastoral or agro-pastoral systems, with no involvement of reproductive technologies (MLFD, 2010). Under these systems natural mating is the only breeding method used: even though its use is characterized by low reproductive performance in terms of conception and pregnancy rates (MLD, 2006; MLFD, 2010). In response there have been and still are continued efforts from government institutions such as Ministry of Livestock and Fisheries Development (MLFD), Tanzania Livestock Research Institute (TALIRI), and Sokoine University of Agriculture (SUA) (MLD, 2006), to improve the reproductive performance and productivity of cows by increase the uptake of reproductive technologies (MLFD, 2010). These efforts include the establishment of the National Animal Biotechnology Laboratory at Tanzania Livestock Research Institute (TALIRI-Mpwapwa) and a National Artificial Insemination Centre (NAIC) in Arusha, alongside the development of a National Livestock Policy (NLP), and a National Breeding policy (MLD, 2006; MLFD, 2010).

A number of trials and experiments have been conducted by these government institutions to investigate the effectiveness of using AI and ET to improve the reproductive performance and productivity of beef and dairy cows (MLFD, 2010). At the moment, there is limited published information on the results of these ongoing studies of reproductive technologies other than interim reports that suggest that, particularly in beef cattle, results have not been as good as hoped. Even in dairy cattle, progress has been slow. Simple programmes which facilitate the effective use of AI are needed in beef cattle.

Chapter 2

Literature Review

Economics of Beef Production in Tanzania

Tanzania Beef Industry

The Tanzania beef industry is mainly composed of three components: Multiplication Livestock Units (MLU), the National Ranching Company (NARCO) and individual small-scale farmers (MLFD, 2012). Beef cattle reared on MLUs and NARCO farms used to be operated and owned by the government (MLFD, 2012). However, currently these farms operate under a public private partnership (PPP) whereby some portions of these farms are operated by private companies to try and counter the production inefficiencies associated with government farms (MLFD, 2012). Examples of MLU farms include: Mabuki farm in the Simiyu region, Nangaramo in Mtwara and Sao hill farms in the Iringa region, Ngerengere farm in Morogoro (MLFD, 2012), together with Kongwa ranch in Dodoma, Mzeri ranch in Tanga, Ruvu ranch in the Coastal region, Mkata ranch in Morogoro, Kikulula and Missenyi ranches in Kagera, West Kilimanjaro ranch in Kilimanjaro, and Kalambo ranch in Rukwa NARCO ranches (MLFD, 2012). MLUs and NARCO farms have been placed in different agro-ecological zones of the country mainly to accommodate the native cattle eco-types or breeds available in the areas of their locality (MLFD, 2012). Table 5 and 6 summarises the production potentials of farms and ranches under MLUs and NARCO respectively.

Table 5 Production potential of farms under MLUs

S/No	Farm	Size (ha)	Region	Eco-type	Cattle Present	Carrying Capacity
1	Mabuki	9,793	Simiyu	Boran, TSZ	2,684	6,000
2	Sao Hill	6,500	Iringa	Boran and its crosses	1,759	3,000
3	Nangaramo	6,175	Mtwara	Boran and its crosses	441	4,000
4	Ngerengere	4,562	Morogoro	Boran and its crosses	574	3,500

Source: (MLFD, 2012)

Table 6 Production potential of ranches under NARCO

S/No	Ranch	Size (ha)	Region	Eco-type	Cattle Present	Carrying Capacity
1	Kongwa	38,000	Dodoma	Boran, TSZ	8,032	100,000
2	Mzeri	21,236	Tanga	TSZ, Boran	3,400	7,080
3	Ruvu	43,000	Coastal	TSZ, Boran	3,762	10,238
4	Mkata	19,446	Morogoro	TSZ, Boran	0	7,000
5	Kikulula	42,083	Kagera	Ankole, TSZ	6068	21,030
6	Missenyi	23,998	Kagera	Ankole, TSZ	7,309	9,599
7	W.Kilimanjaro	19,910	Kilimanjaro	TSZ, Boran	967	8,000
8	Kalambo	23,588	Rukwa	TSZ, Boran	1,733	8,730

Source: (MLFD, 2012)

*TSZ, Tanzania Shorthorn Zebu (Table 5 and 6)

Current Status of Beef Production in Tanzania

The main beef cattle eco-types farmed under tropical and semi-arid conditions of Tanzania are Tanzania Shorthorn Zebu (TSZ), Boran, Ankole and Mpwapwa (MLFD, 2010, 2012). However, exotic breeds including Brahman, Aberdeen Angus, Hereford, Santa Gertrudis, Simmental, Charolais and Chianina are kept along with indigenous breeds by a few commercial beef farms in the country (MLFD, 2012). Although intensive beef ranching and feedlots have been practiced on ranches managed by NARCO and on some private ranches and farms, beef production in Tanzania is dominated by traditional pastoral and agro-pastoral systems (MLFD, 2012). This traditional beef sector accounts for about 94% of beef production, while that of the intensive beef sector accounts for only about 6% of the total beef production (MLFD, 2010). Nevertheless, although the traditional beef sector is the main contributor to beef production in Tanzania, it is poorly developed, with low reproductive efficiency and productivity (MLFD, 2010, 2012). Furthermore, even though the more intensive parts of the beef system in Tanzania mostly run by NARCO or as MLUs have better reproductive efficiency and productivity than the pastoral/agro-pastoral sectors, even they are under-producing relative to their carrying capacities (MLFD, 2010, 2012). Table 7 summaries the performance of ranches under NARCO.

Table 7 Reproductive and Production Performance of Ranches under NARCO

S/NO	Ranch	Conception rate (%)	Calving rate (%)	Mortality rate (%)	Weaning rate (%)	Off take rate (%)
1	Kongwa	90	85	1.5	75	25
2	Mzeri	80	70	2	63	17
3	Ruvu	80	70	2	75	17
4	Mkata	80	71	2	65	14
5	Kikulula	-	51	2	75	28
6	Missenyi	80	80	2	70	29
7	W. Kilimanjaro	80	90	2	75	22
8	Kalambo	65	80	2	76	38

Source: (MLFD, 2012)

Constraints facing Beef Production in Tanzania

The performance of the Tanzanian beef industry in the previous three decades have shown a number of significant achievements which in turn have positively contributed to the growth of livestock sector as a whole in the country (MLFD, 2011). However, despite of all the achievements and success brought by the beef industry in the livestock sector, beef production in Tanzania has encountered several constraints and challenges. These include:

a) Grazing land development, management, and utilization.

Shortage of grazing land is currently posing a high risk to the sustainability of the livestock sector, with frequent conflicts occurring between pastoralists and crops farmers especially in areas with large number of cattle and other livestock species (MLFD, 2010, 2011). This shortage of grazing land is a result of increased population of both livestock and humans in the country (MLFD, 2011). This has been exacerbated by expansion of both national parks and game reserves areas for wildlife conservation which has also caused significant reduction of livestock grazing areas and range lands (MLFD, 2010, 2011).

b) Pastures and pasture seeds availability.

The available natural pastures within the range lands are not sufficient to sustain the increased population of livestock in the country. In addition, the quality of these pastures tends to fluctuate within and across seasons, especially during the dry season (MLFD, 2011). The lack of pasture and pasture seed availability within the grazing and range lands is due to inadequate knowledge of farmers about quality pasture production, pasture seed production, and forage conservation (MLFD, 2011).

c) Reliability and availability of water sources.

Regions that have a large number of livestock have experienced frequent conflicts between human and livestock use of water resources (MLFD, 2011). The most affected regions are those that have a large number of livestock and are located within the dry parts of the country (MLFD, 2010). In most cases, the majority of the water infrastructure developed for livestock is not functional and the minority of functional ones are not sufficient to sustain the number of available livestock in the area (MLFD, 2010, 2011). This situation is as a result of inappropriate and untimely maintenance and refurbishment of these infrastructure (MLFD, 2011).

d) Beef cattle eco-types and breeding methods.

Indigenous beef cattle are well adapted to the tropical environment and are resistant to endemic diseases, but they have poor genetic merit in terms of beef production (MLFD, 2011). Although the poor genetics are the result of the combination of many factors, inbreeding, which is commonly practiced on most Tanzanian beef farms, is the main cause (MLFD, 2011). Most farms use natural mating, with the bulls that are used being home-bred, even though this strategy is linked to poor productivity and reproductive performance (MLFD, 2012). Only a few farms use artificial insemination, mainly in order to increase their productivity (MLFD, 2012).

e) Livestock disease control and prevention.

Considerable work and effort has gone in to control and prevent livestock diseases by the veterinary authorities within the country (MLFD, 2011, 2012). However, further efforts and more control measures are still required to better tackle livestock disease outbreaks (MLFD, 2011). The inability to completely control livestock diseases that cause harm to humans especially transboundary animal diseases, has for several years been the main challenge to international trade and been an important impediment to progress of beef production in Tanzania (MLFD, 2011).

f) Livestock extension services.

   The availability of extension services to livestock farmers is still inadequate in most of the areas in the country (MLFD, 2011). Lack of good sources of information for livestock owners has led to the persistence of poor husbandry and reduced production increases. Government efforts to increase the number of livestock extension are ongoing but are hampered by the lack of learning centres for farmers and the absence of farmer-led groups such as unions (MLFD, 2011, 2012).

g) Livestock markets

   Many farmers do not have access to livestock market information on a consistent basis. This means that farmers cannot make informed decisions as to when they should take their livestock to the markets (MLFD, 2010). This often results in reduced to already poor farmers, which means they lack the funds to improve their livelihood and management of their livestock (MLFD, 2012).

OESTROUS CYCLE IN CATTLE

The oestrous cycle refers to the regular periods or cycles between reproductive receptivity and non-receptivity of female mammals (Forde et al., 2011). Cattle are nonseasonally polyoestrus (i.e. they cycle all through the year), with an average length of 21 days per cycle. The oestrous cycle of cattle consists of follicular and luteal phases which last, on average, for 18 and 3 days respectively (Forde et al., 2011). The follicular phase is initiated by regression of the corpus luteum (luteolysis), which permits a dominant follicle to mature to the pre-ovulatory stage. Follicular growth is terminated by ovulation, following which the remnants of the follicle are transformed into the corpus luteum. Oestrus and ovulation are the most important and visible events in the oestrous cycle, but these are themselves dependent upon the regression of the corpus luteum of the previous cycle (Forde et al., 2011).

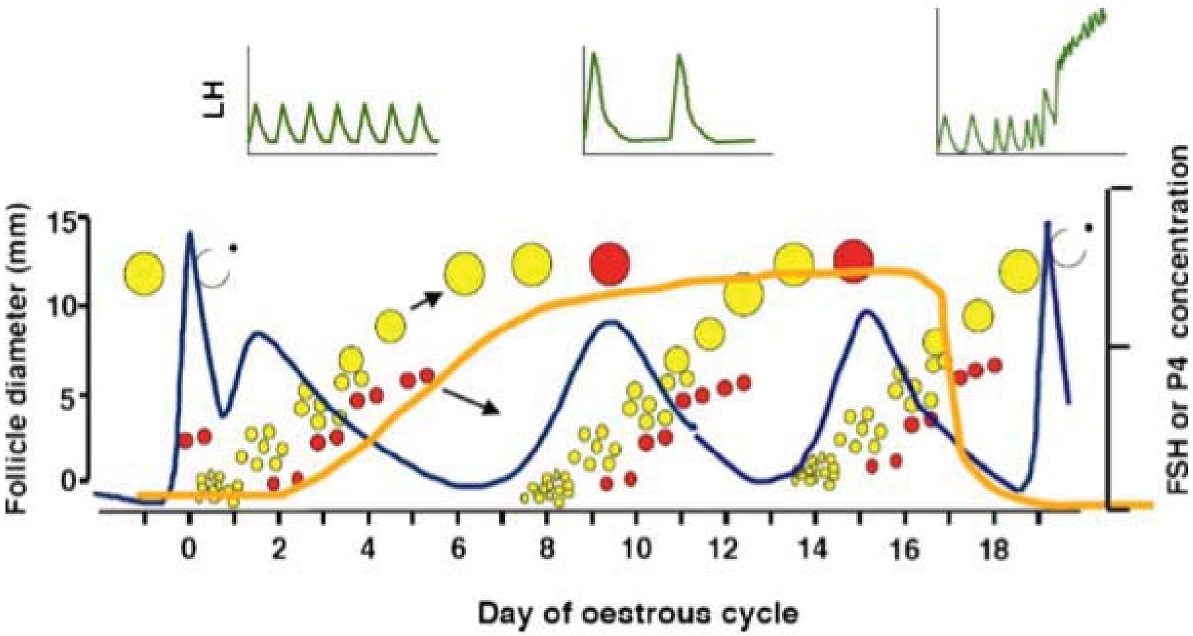
Much work has been done in developing our understanding of the physiology of the bovine oestrous cycle, with ultrasonography and hormonal assays being particularly important as they provide complementary information (Sartori and Barros, 2011). Whilst most attention has been given to *B. taurus* cattle, recent years have seen a sharply increased focus upon *B. indicus* (Sartori and Barros, 2011).

Hormonal control of oestrous cycle

The control of the oestrous cycle is coordinated by the following hormones: GnRH, secreted by the hypothalamus gland; FSH and LH, secreted by the anterior pituitary gland;

progesterone, oestradiol and inhibin, secreted by the ovaries, and $F_{2\alpha}$, secreted by the uterus (Forde et al., 2011). The co-ordinated activity of these hormones maintains the oestrous cycle through the operation of several balanced feedback mechanisms (Roche, 1996).

Figure 1 Hormonal control of oestrous cycle; relationship with follicular growth



Key: FSH (blue line); progesterone (orange line); LH (green inserts); normally developing follicles (yellow); inferior follicles (red). Source: (Forde et al., 2011)

Follicular waves

In cattle, follicular growth and its regression follows a wave-like pattern typically with usually two to three waves per cycle (Forde et al., 2011; Figure 3). Wiltbank et al. (2011) defined the onset of a follicular wave as follicular growth which exceeds the diameter of 4 mm after the selection of the dominant follicle; this marks the dominance of the largest or dominant follicle and growth rate reduction of the smaller or inferior follicles (a process known as follicular deviation; Starbuck et al., 2007; Wiltbank et al., 2011).

Hormonal involvement in follicular waves

Follicular waves are initiated and regulated by the hormones of the hypothalamo-pituitary-gonadal axis (Smith, 1986; Wiltbank et al., 2011). Primary control is exercised by the hypothalamic neurosecretory hormone, GnRH, which acts upon the gonadotrophin-producing cells of the anterior pituitary. Production of FSH is stimulated by the secretion of GnRH. Homeostatic control of FSH secretion relies upon the secretion of oestradiol and inhibin from the granulosa cells of mature follicles: oestradiol exerts its action by negative feedback upon

GnRH secretion, whilst inhibin directly suppresses the ability of the pituitary to secrete FSH in response to GnRH (Wiltbank et al., 2011). Secretion of LH is also regulated by GnRH. Negative feedback control on LH is principally exercised through progesterone, which causes a low-frequency/high amplitude episodic pattern of LH secretion throughout the luteal phase. Additionally, low concentrations of oestradiol also cause negative feedback on LH (especially when accompanied by moderate/low concentrations of progesterone), but, in general, as progesterone concentrations decline, the frequency of episodes of LH secretion rises, causing a net rise in its concentration. High concentrations of oestradiol cause the generation of the preovulatory GnRH/LH surge, which is responsible for final follicular maturation and ovulation. The LH surge is accompanied by an FSH surge - and a second FSH surge that occurs 24 h later. This second FSH surge is regarded as being the initiator of the first follicular wave that occurs during subsequent luteal phase (Bergfelt et al., 2000).

Follicular dynamics

As discussed earlier each oestrous cycle in cattle has either two or three follicular waves that result in the formation of a dominant follicle, whilst the remainder of the cohort, progressively undergo atresia (Noseir, 2003; Forde et al., 2011; Bridges et al., 2014), with the first follicular wave starting 24 h after the second FSH surge of the ovulatory process (Smith, 1986; Forde et al., 2011; Bridges et al., 2014). The second follicular wave starts on the ninth or tenth day in a 2-wave cycle, and between the eighth and ninth day in a 3-wave cycle (Burns et al., 2005; Forde et al., 2011; Bridges et al., 2014), with the third follicular wave developing on the fifteenth or sixteenth day (Forde et al., 2011; Bridges et al., 2014).

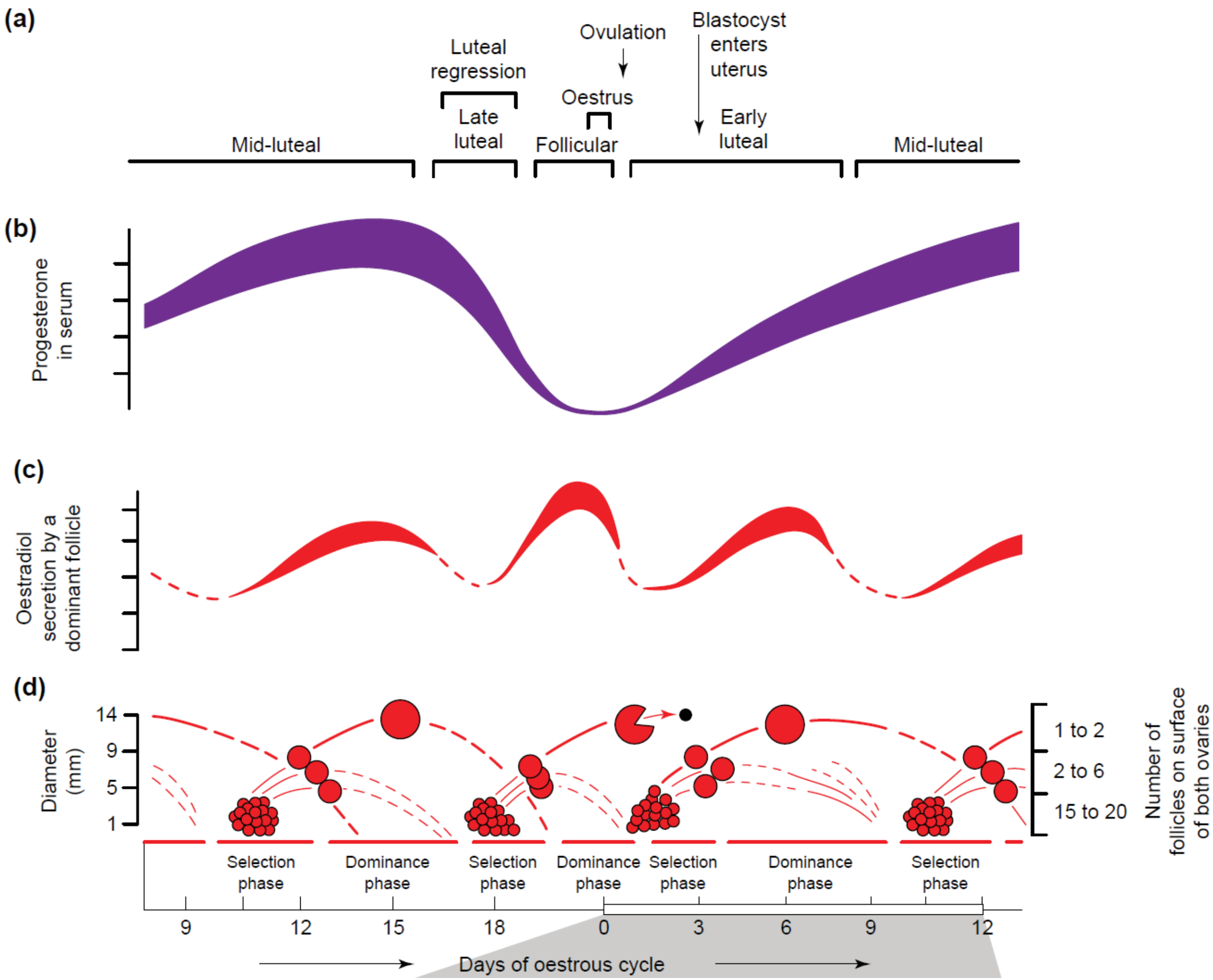
The duration of the oestrous cycle is different between 2-wave and 3-wave cattle, being 18–20 days in 2-wave cows and 23-24 days in 3-wave cows (Forde et al., 2011; Bridges et al., 2014).

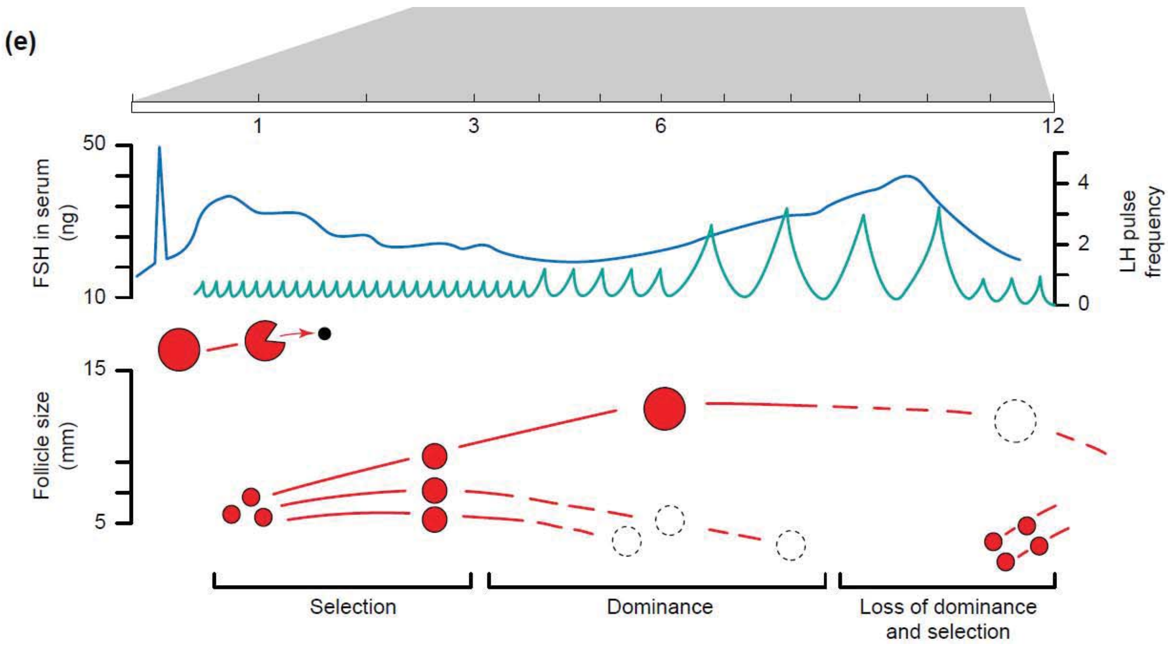
Recruitment of follicular waves and selection of a dominant follicle is established by differential responsiveness to FSH and LH (Bergfelt et al., 2000; Forde et al., 2011; Atkins et al., 2013). Surges in plasma FSH concentrations stimulate the development of a follicular wave, but FSH is afterwards blocked or inhibited by products of the growing follicles (oestradiol and inhibin) (Bergfelt et al., 2000; Forde et al., 2011; Atkins et al., 2013). As a result, the concentration of FSH is decreasing when growth profiles of the dominant and subordinate follicles are at their most dynamic (i.e. during the time of selection) on a third day after wave development (Bergfelt et al., 2000; Forde et al., 2011; Atkins et al., 2013).

Within each wave, the follicle that develops into a dominant follicle is also the first to obtain LH receptors, whereas subordinate follicles still need FSH to sustain growth and to prevent them from undergoing atresia (Bergfelt et al., 2000; Forde et al., 2011). Then, the dominant follicle grows for around six days and finally arrives in a static phase (Bergfelt et al., 2000; Forde et al., 2011; Atkins et al., 2013).

On the other hand, the loss of FSH coupled with a progesterone-induced suppression of LH that causes the dominant follicle to falter in its metabolic functions after two or three days, and it begins to regress. FSH concentrations then again rise (Forde et al., 2011; Atkins et al., 2013). Finally, the ovarian cycle then restarts itself, and it is clear that follicle-wave dynamics have an influence on the efficiency of oestrus synchronization programs (Smith, 1986; Forde et al., 2011).

Figure 2 Follicular wave dynamics





a) Stages and events in luteal phase; b) change of progesterone concentration; c) change of oestradiol concentrations; d) size and number of dominant and inferior or poor follicles in different phases; e) change of FSH and LH concentrations and their effect on follicle size in different phases.
Source: (Roche, 1996).

Follicular wave dynamics are characterized by three main phases:

Recruitment phase:

The recruitment phase is the phase during which the growth of follicular waves is initiated by an increase in circulating FSH concentrations (Pursley et al., 1996; Wiltbank and Pursley, 2014). The recruited follicles grow as a cohort, their growth is FSH-dependent (Pursley et al., 1996; Wiltbank and Pursley, 2014).

Selection phase:

The selection phase is the second phase of follicular development. For reasons not yet well understood; only one dominant follicle is selected from the group recruited by the small increase in follicle stimulating hormone (Pursley et al., 1996; Wiltbank and Pursley, 2014). Besides, a defining characteristic of the dominant follicle seems to be its greater capacity for oestradiol production (Pursley et al., 1996; Wiltbank and Pursley, 2014). Selection of the dominant follicle is related to the decrease in FSH, and the subsequent maintenance of FSH concentrations at basal values (Pursley et al., 1996; Wiltbank and Pursley, 2014). Thereafter, the selected future dominant follicle develops LH receptors that permit it to continue grow in the environment of low FSH and increasing LH concentrations (Pursley et al., 1996; Wiltbank and Pursley, 2014). It seems that, by decreasing the FSH support, the selected follicle uncouples subordinate follicles from their vital growth stimulant (Pursley et al., 1996; Wiltbank and Pursley, 2014). In other words, the selected follicle benefits from both the low FSH environment and increasing LH stimulation (Pursley et al., 1996; Wiltbank and Pursley, 2014).

Dominance phase:

This is the last phase during which the oestrogenic activity and lifespan of the dominant follicle are controlled by the LH pattern (Pursley et al., 1996; Wiltbank and Pursley, 2014). Furthermore, in response to the oestrogens produced by the dominant follicle, once concentrations exceed a critical threshold, a massive LH release takes place (Pursley et al., 1996; Wiltbank and Pursley, 2014). As it acts as a direct stimulation of ovulation, it is therefore termed as a pre-ovulatory luteinizing hormone surge or peak (Pursley et al., 1996; Wiltbank and Pursley, 2014).

Relationship between the timing of luteolysis and the timing of oestrus

The timing of luteal regression and thus the length of the oestrous cycle is principally determined by the timing of $PGF_{2\alpha}$ release by the endometrium. Spontaneous luteolysis in cattle is initiated by a feedback loop between oxytocin (Flint and Sheldrick, 1983) and endometrial $PGF_{2\alpha}$. This process is mediated by the development of oxytocin receptors in the luminal and glandular endometrial epithelium in the late luteal phase, as it is the binding of oxytocin receptor which results in the release of $PGF_{2\alpha}$ by the endometrium. Oxytocin receptor numbers increase during this period in response to changing oestrogen and progesterone concentrations (Mann et al., 2001; Mann and Lamming, 2006). Oxytocin is released by the corpus luteum throughout the luteal phase but at the end of this phase, a positive feedback loop develops as the release of uterine $PGF_{2\alpha}$, stimulates further luteal oxytocin secretion, until the pool of releasable oxytocin is depleted (Flint and Sheldrick, 1985; Mann and Lamming, 2006). Four such episodes of $PGF_{2\alpha}$ secretion in a 24-hour period are required for luteolysis (McCracken et al., 1973). The $PGF_{2\alpha}$ is transferred to the ovary from the uterus via a counter-current pathway involving the utero-ovarian vein and the ovarian artery (Ginther et al., 2007).

Luteolysis can also be caused by exogenous administration of $PGF_{2\alpha}$ (or an analogue). In this case, neither the oxytocin feedback loop nor the vascular transfer system seem to be required for luteolysis to occur (Hansel, 1975). For exogenously administered $PGF_{2\alpha}$, the interval between the completion of luteolysis and the time of ensuing ovulation is determined by the stage of the follicular wave when the $PGF_{2\alpha}$ is given (Kastelic et al., 1990). In principle, the further developed the follicle in terms of dominance/size, the shorter the interval to ovulation. However, this relationship is complicated by the 'post maturity' of large, aged, dominant follicles, whose fertility at FTAI appears to be poorer than that of follicles that had been dominant for a shorter period of time (Ireland, 2000).

OESTRUS SYNCHRONIZATION USING PROSTAGLANDIN

PGF2α and related products were recognized, characterized, and manufactured during the 1970s (Lauderdale, 2005). $PGF_{2\alpha}$ was shown to be luteolytic in cattle in 1972 and soon after used to synchronize oestrus (Lauderdale, 2005).

When oestrus synchronization is managed properly, it is an effective reproductive management tool that aids the use of artificial insemination (Stevenson et al., 2000; DJarnette et al., 2001; Lamb et al., 2006). However, it should not be used as a substitute for appropriate nutrition or high quality herd health-programs, nor should it be used as a support for poor management (Stevenson et al., 2000; DJarnette et al., 2001). Selection of candidates for synchronization in an important part of the process and significantly improves response rates (Stevenson et al., 2000; Lamb et al., 2006). For example, oestrus synchronization is easier to accomplish with heifers than cows as they have less metabolic demands on them (e.g. they are not lactating) (DJarnette et al., 2001; Lauderdale, 2010).

$F_{2\alpha}$ plays a crucial role in the regulation of the oestrous cycle, which is it why can be a useful tool for synchronizing oestrus. However, it is not suitable in all situations, First, prostaglandin does not synchronize oestrus unless a corpus luteum is present (Stevenson et al., 2000; DJarnette et al., 2001; Lauderdale, 2005). Thus prostaglandin is not useful in cows that are in anoestrus, or in pre-pubertal heifers or in cyclic cows which are not in the luteal phase. Secondly - prostaglandin does not affect follicular waves (Stevenson et al., 2000; DJarnette et al., 2001; Lauderdale, 2005), so the time between treatment with prostaglandin and oestrus/ovulation is dependent on the timing of injection relative to where the dominant follicle is in its follicular wave.

Cattle with large follicles tend to show oestrus within 1-2 days of injection, while those with smaller follicles can take 3-4 days (Stevenson et al., 2000; DJarnette et al., 2001; Lauderdale, 2005). This variability in onset of oestrus (and, subsequently, ovulation) means that fixed-time artificial insemination following synchronization of oestrus with $PGF_{2\alpha}$ - alone does not produce satisfactory conception rates (Stevenson et al., 2000; DJarnette et al., 2001; Lauderdale, 2005). Nevertheless, prostaglandin-only programs are very effective management tool that can be used to reduce the labour and expense of artificial insemination as well as the period of heat detection (Stevenson et al., 2000; DJarnette et al., 2001; Lauderdale, 2005). Alternatively, other hormones can be used alongside PGF-based systems to improve oestrus synchronization and subsequent reproductive outcomes. (Stevenson et al., 2000; DJarnette et al., 2001; Lauderdale, 2005).

Prostaglandin F_{2a}-based breeding platforms (programs)

PGF_{2a} – based programmes are generally based around a two injections: groups of cows are all treated with prostaglandin, oestrus detection is initiated and then cows are mated to a detected oestrus. Cows that are not inseminated in response to the first treatment are then re-treated with prostaglandin 11-14 days later, with cows inseminated again to observed oestrus or inseminated using FTAI (Stevenson et al., 2000). Therefore, an arrangement of PGF_{2a} treatment two weeks prior to the first mating, assist the improvement of oestrus reaction during the first mating chance (Stevenson et al., 2000).

Prostaglandin-based breeding is simple, inexpensive, and easy to schedule and implement (Lauderdale, 2010). Prostaglandin-based programs provide flexibility if circumstances require, as moving the arrangement of injections by one or two days, has only limited impact (Lauderdale, 2010).

On the other hand, in addition to a lack of response in cows without a CL, $PGF_{2\alpha}$ – based programs are not effective during the first 6–7 days of the oestrous cycle even if the cow has a functional CL (Lauderdale, 2010). The requirement for heat detection means that prostaglandin-based programs work best when heat detection is good and in cows with normal oestrus behaviour (Lauderdale, 2010), or when used entirely with FTAI.

Methods of synchronizing follicular waves using prostaglandin

PGF Methods

i. Seven days of oestrus observation are followed by $PGF_{2\alpha}$ injection to cows which failed to display oestrus, followed by FTAI 72 and 96 hours later.
ii. An 11-day protocol based on two $PGF_{2\alpha}$ injections 11 days apart. After the first injection, oestrus detection can be used and cows mated to observed oestrus; after the second FTAI can be used 72-84 hours later (if a single AI is used) or 72 and 96 hours later if two FTAI are used.
iii. A 14-day protocol, which is similar to the 11-day, but the two injections are separated by 14 days. The 14 days protocol has better response to FTAI than the 7 or 11 day

protocols, as it enables most cows at the second $PGF_{2\alpha}$ injection without oestrus detection to be in the luteal phase (Lauderdale, 2010).

Simple PGF and progesterone method

The issue with non-responsiveness to prostaglandins in the earlier luteal phase can be overcome by combining an intra-vaginal progesterone device (e.g. CIDR) with the prostaglandin injection (Lucy et al., 2001; Lauderdale, 2010). A short duration of progesterone supplementation is used (usually 7 days), with a $PGF_{2\alpha}$ injection given either the day before, or on the day of, device removal (Lucy et al., 2001; Lauderdale, 2010). The use of progesterone device results in suppression of ovulation, and in cows without a CL its removal results in ovulation at the same time as ovulation induced by luteolysis in those with a CL. (Lucy et al., 2001; Mapletoft et al., 2003; Lauderdale, 2010).

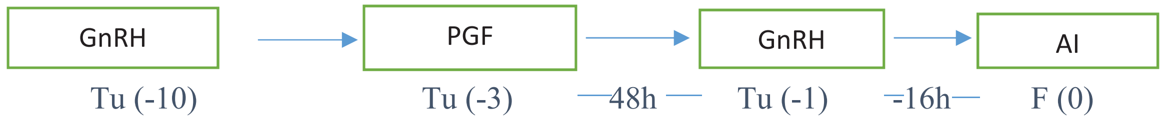
Ovsynch and related methods

In these programs, GnRH is used to, firstly, increase the proportion of cows at the right stage in their follicular wave when the prostaglandin is given and, secondly, to tighten the timing of ovulation after the prostaglandin injection. In Ovsynch, the first GnRH injection, which ovulates late stage dominant follicles, is given 7 days before the $PGF_{2\alpha}$ injection, and the second GnRH injection is administered 2 days afterwards. FTAI is then performed 16-24 hours after the second GnRH injection (Kesler, 2005; Ion, 2013). This is mostly used in dairy cows (Lean et al., 2003; Kesler, 2005). Co-synch, which involves FTAI at the time of the second GnRH injection, is commonly used in beef cows. Select-synch, in which only the first GnRH injection is given, with AI on oestrus observation rather than FTAI following the $PGF_{2\alpha}$ injection (Patterson et al., 2007), is also commonly used in beef cows in order to reduce the number of times the animal has to be handled.

Reasons for choosing PG as synchronization method of my choice

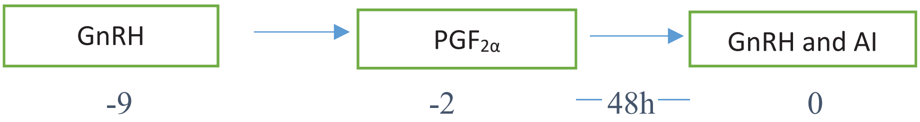
PG-based programmes are very effective management tools in the sense that they are simple (easy to schedule and implement) and inexpensive (reduce the cost of labour and expense of AI as well as the time of heat detection). On the other hand, PG-based programmes provide flexible environment for adjusting the arrangement of injections by one or two days. Similarly, PG-based programmes work best when used entirely with FTAI particularly when heat detection is good and in cows with normal oestrus behaviour. Lastly, PG-based programmes can work on *Bos. Indicus* cattle.

Figure 3 Ovsynch protocol



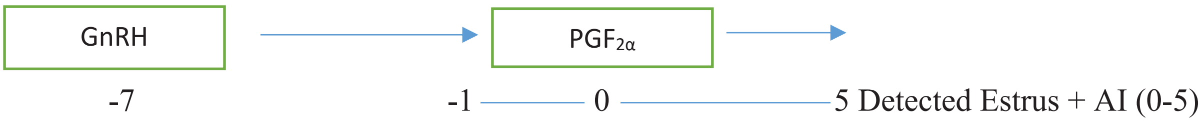
Source: (Kesler, 2005)

Figure 4 Co-synch protocol



Source: (Kesler, 2005)

Figure 5 Select-synch protocol

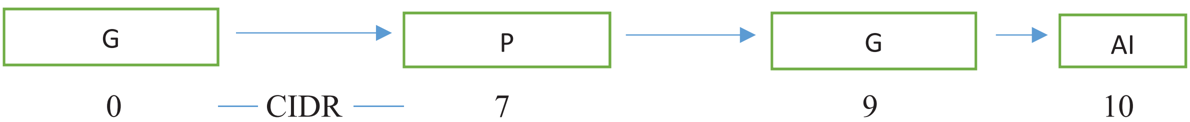


Source: (Kesler, 2005)

Ovsynch + Progesterone method (Ovsynch + CIDR/Progesterone protocol)

Progesterone devices can be added to Ovsynch protocol; they are particularly useful in cattle which are in apparent anoestrus. The protocol is exactly the same as for Ovsynch except that a progesterone device is inserted on the same day as the first GnRH and removed on the same day as the prostaglandin injection. Both Ovsynch and Co-synch can be used with a progesterone device (Bó, et al., 1995; Bó, et al., 2003).

Figure 6 Ovsynch + Progesterone/CIDR



Source: (Lucy, 2007)

Key: G = GnRH; P = $PGF_{2\alpha}$

CATTLE FERTILITY

Cows

Factors affecting cow' fertility during the breeding season

a) Nutrition

Good balanced nutrition which meets cows' energy, protein and mineral needs is essential for good fertility (Mass, 1987). In cattle, poor nutrition has been associated with a wide range of reproductive disorders including anoestrus, silent oestrus, delayed ovulation, conception failure, and increased embryo mortality, ovarian cysts and infertility (Mass, 1987). Of all the nutritional issues, insufficient energy intake (relative to output) is the most important factor in producing poor fertility (Mass, 1987).

b) Disease

Disease is the second in importance to nutrition in term of fertility. Disease can directly affect fertility by impacts on the reproductive tract or the developing embryo (e.g. Campylobacter, IBR and BVD), or through indirect impacts whereby affected cattle are less likely to show oestrus or have normal oestrous cycles (e.g. mastitis, lameness) (Bath, 1993).

c) Season

Although cattle are seasonally polyoestrus, this does not mean that they are equally fertile throughout the year, this seasonality is mainly due to nutrition. Particularly in tropical climates there is significant seasonal variations, particularly in regards to anoestrus. Cows in seasonal anoestrus are unable to secrete LH to support the growth of the developing follicle into maturation stages hence cause cows' infertility (Mass, 1987).

Bulls

In order for a bull to become fertile and to attain maximum performance, it has to possess the following components of bull fertility such as libido and physical fitness, ability to achieve intromission and deposition of semen into the vagina, producing semen of enough quantity and high quality, and lack of disease transmission to the cows during the breeding season (Parkinson, 2004).

BSE is a rapid and low cost method of assessing the breeding ability or capability of bulls (Parkinson, 2004; Godfrey and Dodson, 2005). BSE classifies bulls into three main groups: i) satisfactory potential breeder, ii) questionable breeder and iii) deferred or unsatisfactory (Parkinson, 2004; Godfrey and Dodson, 2005). The key steps of a BSE are: i) visual assessment of the feet, legs, eyes, teeth and external genitalia, ii) palpation of the seminal vesicles, iii) measurement of the scrotum and palpation of the testis and epididymis, iv) collection of semen sample for microscopic evaluation (Parkinson, 2004; Godfrey and Dodson, 2005; Kastelic and Thundathil, 2008; Kastelic, 2013; Silva et al., 2014).

Table 8 Rejection percentages of young and mature bulls according to breeding soundness evaluation (BSE) steps.

Steps/examination		Rejected/evaluated (%)		Rejected%	Probability
		Young	Mature		
General physical	Year I	78/3131 (2.5)	252/3743(6.7)	4.8	<0.0001
Genital		210/3053(6.9)	326/3491(9.3)	8.2	0.0003
Semen		50/2843(1.8)	78/3165(2.5)	2.1	0.0586
Behavioral		91/1507(6.0)	78/2115(3.7)	4.7	0.0009
General physical	Year II	19/2155(0.9)	201/4765(4.2)	3.2	<0.0001
Genital		108/2136(5.1)	391/4564(8.6)	7.4	<0.0001
Semen		51/2028(2.5)	143/4173(3.4)	3.1	0.5030
Behavioral		24/1240(1.9)	112/2881(3.9)	3.3	0.0013
General physical	Year III	52/2625(1.9)	255/5694(4.5)	3.7	<0.0001
Genital		200/2573(7.7)	399/5439(7.3)	7.5	0.4873
Semen		126/2373(5.3)	277/5040(5.5)	5.4	0.7414
Behavioral		20/915(2.2)	49/1681(2.9)	2.7	0.2699

Source: (Menegassi et al., 2012)

The % of general physical parameters between mature and young bulls is lower, with no consistent differences for other parameters. However, general physical parameters and other parameters such as genital, semen and behavioural as summarised in Table 8 have shown that most mature bulls tend to be rejected from year I to III as compared to young bulls.

Fertility factors examined or assessed during breeding soundness evaluation are as describe below;

a) Mating ability

This refers to the physical ability or potential of bulls to breed a cow effectively and efficiently (Perry et al., 2008). In order for a bull to achieve this it must be able to see, eat, smell and move normally there needs to be no diseases or injuries of bulls' penis or prepuce. (Perry et al., 2008).

b) Scrotal circumference

Scrotal circumference is strongly correlated with daily sperm production and fertility rates (Perry et al., 2008). The very strong relationship between scrotal circumference and puberty means that measuring scrotal circumference is particularly useful for evaluating yearling bulls (Parkinson, 2004); McGowan et al., (2002) found that 52 % of bulls with a scrotal circumference of 28 cm were found to be in pubertal, whereas 97 % of bulls attain puberty once their scrotal circumference measures 30 cm. After puberty is reached, scrotal circumference continues to increase until it measures between 33 and 34 cm once bulls are >2 years old (Parkinson, 2004). Heritability of mature scrotal circumference is very high (Quirino and Bergmann, 1998).

c) Semen quality

Assessment of semen quality is based on volume of ejaculate, alongside sperm motility and morphology. Key factors which reduces semen quality poor plane of nutrition, high environmental temperatures, and disease (Fitzpatrick et al., 2002; Perry et al., 2008).

Sperm abnormalities are classified as either major defects (such as abnormalities of the head, mid-piece, and proximal cytoplasmic droplets) or minor defects (such as abnormalities of the tail, distal cytoplasmic droplets) (Blom, 1950).

Table 9 Bulls were selected randomly or had more than 80% normal sperm cells. All bulls had a scrotal circumference of more than 32cm and successfully passed a breeding soundness evaluation (BSE) test

	Year 1		Year 2	
	Random Group	≥80% Normal Sperm	Random Group	≥80% Normal Sperm
Cows Exposed	655	675	1282	808
# of bulls	26	27	51	33
# pregnant	571	656	1179	769
% pregnant	87%	93%	85%	90%
% increase		6%		5%

Source: (Wiltbank and Parish, 1986)

Table 9 shows that selecting bulls on the basis of having a high proportion of normal sperm can significantly increase fertility, specifically pregnancy rates at the end of the breeding season.

d) Libido

This is the desire of animals to mate. High libido bulls have higher conception rates than low libido bulls (Blockey, 1979). Perry et al., (2008) suggested that, as libido is not associated with scrotal circumference, semen quality or mating ability, but is associated with reproductive outcomes, it should be measured prior to using a bull. However, Parkinson (2004) stated that the performance of bulls in both libido and service examinations was similar. He suggested that quantitative evaluation of libido should be restricted to young or yearling bulls, while proven older bulls could have a qualitative evaluation of libido.

Fertility factors which are not examined or assessed during breeding soundness evaluation are as describe below;

a) Male to female ratio

Recommended bull to cow ratios range from 1:10 to 1:60 (Ellis, 2008; Perry et al., 2008). Libido clearly influences the number of cows an individual bull can mate, but bull ratios are principally affected by their ability to service, the environment the herd is kept in and whether oestrus has been synchronized or not (Perry et al., 2008). Bull age is also crucial with lower ratios recommended for younger bulls.

b) Social dominance

Less dominant bulls perform less well (Parkinson, 2004). The keys factors affecting dominance are age, time spent in the herd, and weight (Parkinson, 2004). Dominance is important as it is not only directly associated with either libido or fertility, so the impact of a sub fertile dominant bull on herd's pregnancy rates can be much greater than that of a lower ranked sub fertile bull (Parkinson, 2004).

Summary and problem statement

Reproductive performance in Tanzanian beef cows is generally low. This poor performance is likely to be multifactorial in origin, with factors such as poor nutrition, insufficient body condition, and reproductive disease all involved. This poor reproductive performance has knock-on effects on productivity and is one of the key reasons why improvements in beef production have not kept pace with improvements in milk production (MLFD, 2010).

Herd fertility management is thus a complex of interlinked issues. Under Tanzanian conditions, the key issues are the generally poor reproductive performance of local cattle breeds and the associated the lack of uptake, particularly in the beef sector, of reproductive technologies, even those as simple as AI. The aim of this study was to evaluate the pregnancy rate at the end of the breeding season of well-managed Mpwapwa cattle which were naturally mated with tested bulls and to compare this with the results from a standard 14-day prostaglandin-programme designed to facilitate the use of AI.

Research hypothesis

Using targeted FTAI can improve reproductive performance (conception and pregnancy rates) of Mpwapwa breed cows.

Research objectives

i. Compare the effects of FTAI and NM on pregnancy rates in Mpwapwa breed cows.
ii. Evaluate the underlying fertility of Mpwapwa breed bulls and cows under controlled conditions.

Chapter 3

Materials and Methods

Study area

This project was conducted at Tanzania Livestock Research Institute (TALIRI-MPWAPWA [TM]) in the Mpwapwa district of the Dodoma region of Tanzania. TM is situated at latitude 26°20'S and longitude 36°30'E in the semi-arid agro-ecological zone of the central part of Tanzania. Its altitude is about 1000 m above sea level. The climate of Mpwapwa district is divided into the dry season, which runs from May to November, and the rainy season, which runs from December to April. The daily mean temperature of the area ranges from 24-29°C, while the total annual rainfall is about 720 mm. The types of soils found in the institute's farm are mainly sandy loams (mainly found on slopes) and clay loams (commonly located at the floor of valleys). Soil pH varies from 5.6 to 7.7 for the top soils and 5.3 to 8.6 for the sub-soils. The grass species grazed by livestock include *Chloris gayana, Themeda spp, Cynodon dactylon* and *Hyperrhenia rufa*.

Figure 7 Rainfall distribution in Mpwapwa district (Source: Tanzania Livestock Research Institute, 2014-2015). This study was undertaken during the rainy season breeding period i.e. March-May.

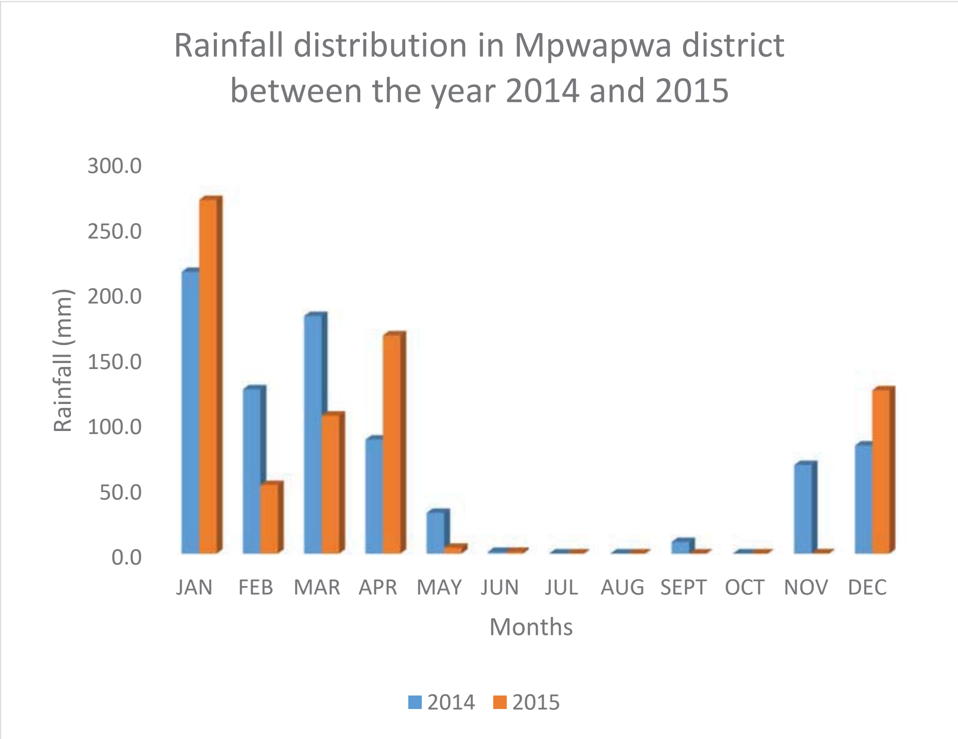
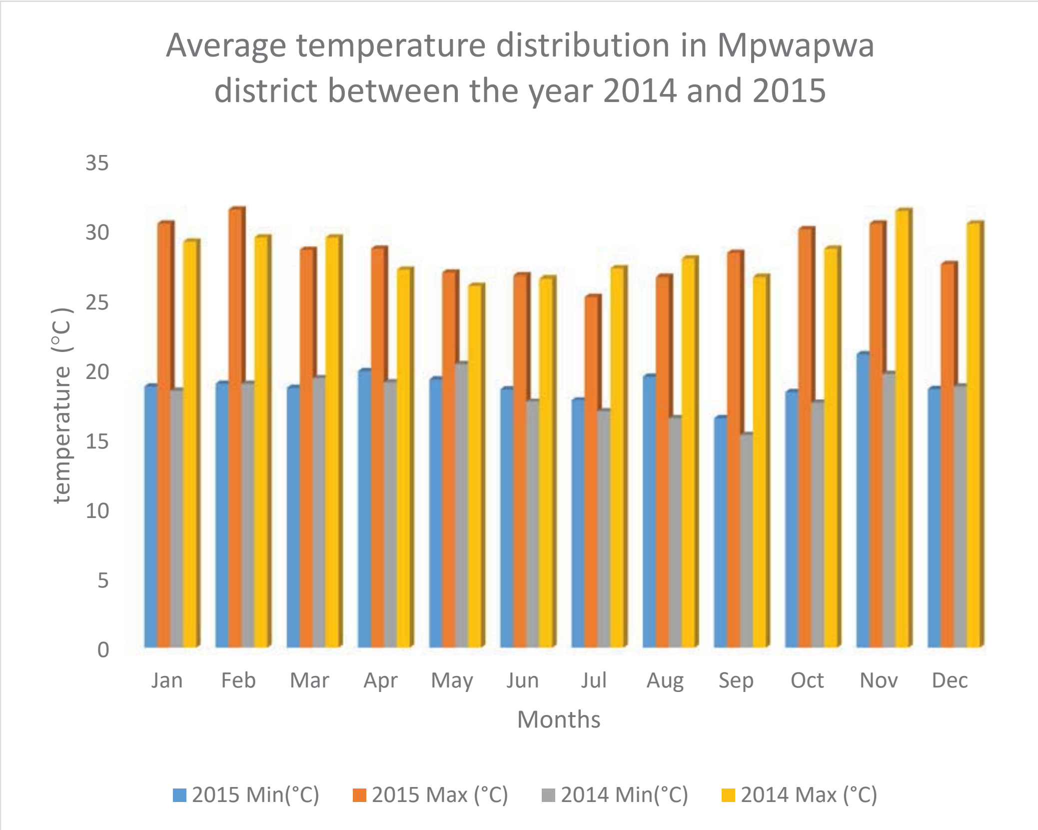


Figure 8 Average temperature distribution in Mpwapwa district (Source: Tanzania Livestock Research Institute, 2014-2015).



Selection of bulls: Breeding Soundness Evaluation

In February 2016, in order to select the 4 bulls needed for the study, Mpwapwa bulls that were ≥3 years old and which had no history of reproductive problems were examined. The testes of each bull were examined for malformation and their scrotal circumference was measured using a flexible tape measure. The mating ability (i.e. physical ability or potential of bulls to breed cows effectively and efficiently) of each bull was then assessed against the following criteria: - i) the ability of bulls to see, eat, smell and move; and ii) absence of injuries of the penis, prepuce, joints and muscles. The four bulls with the largest scrotal circumference of those which had passed the mating ability test were selected and then weighed. Teaser bulls were selected based on their ability of detecting cows on oestrus in a herd.

Selection of cows

In February 2016, pregnancy diagnosis was conducted on all cows present at TM - to identify non-pregnant cows. The 200 non-pregnant cows with the highest bodyweight were then selected for the study (all cows had to be >180kg bodyweight, the minimum used in TM breeding programme). All selected cows were then body condition scored 3 or 4 on the 1–5 BCS scale (Nicholson and Sayers, 1987a, 1987b).

Management of experimental animals

Pre-breeding phase: Grazing and supplementation

To optimize body condition score and health, all cattle were started on a planned nutritional regimen and treatment programme from one month prior to expected start of breeding season (i.e. March, 2016). In addition to unrestricted grazing in selected paddocks, each cow was supplemented using a mineral block (Royal llac, Turkey) containing manganese (manganese oxide 145mg/kg), cobalt (cobalt carbonate 15mg/kg), zinc (zinc oxide 230mg/kg), copper (copper sulphate 162mg/kg), iron (iron oxide 800mg/kg), selenium (sodium selenite 5mg/kg), iodine (calcium iodate 10mg/kg), sodium (sodium chloride 37.6mg/kg), and magnesium (magnesium oxide 0.32mg/kg). The allocation per cow was 1kg of block over the 1-month period. In addition, all cattle were confirmed as negative for brucellosis by testing their serum using rose bengal rapid slide agglutination test (Cho et al., 2010) and treated for intestinal parasites using either 0.5ml/kg of a levamisole/oxyclozanide combination (Nilfarm, Farmers Centre-Tanzania) if lactating, or 50µg/kg ivermectin (Ivermectin, Anglian Nutrition Products-UK) if not lactating. All cattle were dipped once weekly using amitraz (Amitraz 12.5%w/v, Sinochem Ningbo-China) to control ecto-parasites. All cattle were weighed and body condition scored before and after this supplementation period.

Mpwapwa breed cattle photos taken in the farm at TM

Figure 9 Mpwapwa breed cow (A) and Mpwapwa breed bull (B).

A B

Semen straws evaluation, transport and thawing

Two Mpwapwa bulls (KONGWA 27117 and MVUMI 27103) provided the semen for AI. Seventy-five straws of semen from each bull-were purchased from the National Artificial Insemination Centre (NAIC). Prior to purchase, gross motility was tested to confirm semen quality (i.e. >60%) post-thaw motility). The straws were transported in liquid nitrogen from NAIC to TM and then thawed in a water dish at 35°C to 36.2°C prior to insemination. The time between thawing and insemination was ≤2 minutes.

Experimental procedures

On 1st March 2016, the 200 selected cows were grouped into 4 groups of 50 cows based on number order distributed between age and parity, with the first 50 cows in the first group, the second 50 in the second group etc. Mating was staggered for each group (see Table 10). At the start of mating, each group was divided into two treatment groups of 25 cows, again on number order distributed between age and parity:

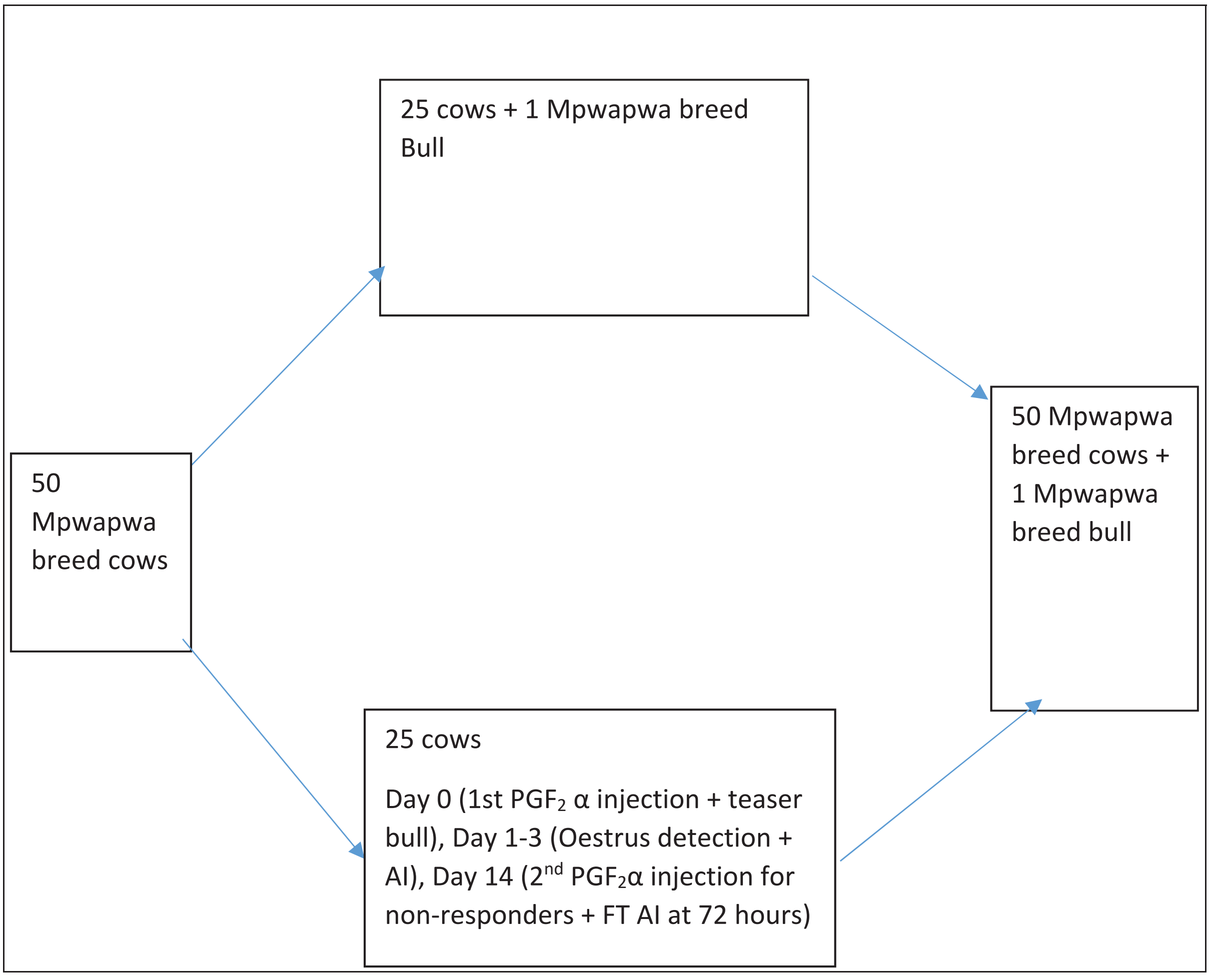
1) Control: NM, with one Mpwapwa breed bull per group of 25 cows; and
2) $F_{2}\alpha$ synchronization and FTAI: on Day 0 each cow received 500µg of cloprostenol (Estroplan, Parnell Australia), with behavioural oestrus observed using one teaser bull per group. Cows seen in oestrus were marked with coloured crayons and inseminated using the purchased semen based on the AM/PM rule (Ion Caraba, 2013). Cows that were not seen in oestrus within 72 hours of the first cloprostenol injection were retreated on Day 14, followed by FTAI at 72 hours after the second cloprostenol injection.

An illustration of the experimental procedures as applied to each of the two treatment groups within each of the four mating groups is illustrated in Figure 14. PD was undertaken for all 4 groups between 26th and 27th of June, 2016 (i.e. on Day 103 to 104 after mating) using transrectal ultrasonography (Chison Medical Imaging Co, China).

Table 10 Timing of key events for each mating group. Cows in group PG were treated with 500 µg of cloprostenol on Day 0 and then 13 days later if oestrus not observed. N/A, not applicable. All cows were pregnancy tested on 26-27/06/16.

	Treatment groups	Day 0	Oestrus detection	2nd PG inj.	Joining of groups
Group 1	PG	16/03/16	17-18/03/16	29/03/16	16/05/16
	Control		N/A	N/A	
Group 2	PG	30/03/16	31/03-01/04/16	12/04/16	30/05/16
	Control		N/A	N/A	
Group 3	PG	13/04/16	14-15/04/16	26/04/16	13/06/16
	Control		N/A	N/A	
Group 4	PG	27/04/16	28-29/05/16	10/05/16	27/06/16
	Control		N/A	N/A	

Figure 10 Simplified diagram illustrating the experimental procedures applied in 4 experimental groups of the project.



Statistical analysis

The data obtained were analyzed using SPSS version 23 (IBM, USA). Chi-square analysis was used to evaluate the effect of pre-breeding treatment on BCS and to compare across treatment groups.

The effect of the two breeding methods on risk of pregnancy was tested by calculating RR with 95% CI, as recommended by (Gardner and Altman, 1990). Available variables including treatment group were then tested in a univariable logistic regression model. All factors where $p<0.25$ were then tested using a multivariable logistic regression model which also included treatment group and interaction between the selected variables and treatment group.

Chapter 4

Results

Bull evaluation

All 14 bulls which were tested had good mating ability; their SC ranged from 21 to 33 cm. The results of the four chosen bulls along with their age, and pre-and post-breeding weights are summarized in Table 11.

Table 11 SC of the four selected bulls used in the study.

Group No	Age (years)	SC (cm)	Pre-breeding weight (kg)	Post-breeding weight (kg)	No. of cows conceived	No. of cows failed to conceive
1	5	30	307	305	10	15
2	12	30	335	330	13	12
3	5	33	325	315	14	11
4	3	30	240	235	12	13

Table 12 Gross motility results after testing 75 semen straws of each of the two Mpwapwa breed bulls.

Name of bull	No. semen straws purchased	No. semen straws tested	Gross Motility
KONGWA 27117	75	5	>60%
MVUMI 27103	75	5	>60%

Table 12 shows the gross motility of 10 semen straws, 5 out of 75 straws from each bull tested at the National Artificial Insemination Centre at Usa river in Arusha.

Cow evaluation

All 200 cows used in the study scored 3 or 4 on the 1–5 BCS scale; the proportion of cows in BCS 4 increased during the preparatory month (X^2=33.2, p<0.001).

Table 13 BCS results for the 200 study cows one month prior to and at the start of the breeding season.

BCS	No. cows at pre-pre-breeding weight (n)	No. cows at pre-breeding weight (n)
3	113	56
4	87	144

No difference between BCS in FTAI and NM groups (P>0.5). (i.e. 3 (56.5% and 28%) and 4 (43.5% and 72%) for BCS scored on cows at pre-pre-breeding weight and pre-breeding weight respectively) as shown in Table 13 above.

Table 14 Descriptive data for the four groups of cows used to compare NM with FTAI. Within each group there was no difference between the two treatment groups in any of these factors. (P >0.25).

	Treatment Groups	Age (years)	Parity	Last calving (days)	Pre-pre-breeding weight (kg)	Pre-breeding weight (kg)	BCS before	BCS after
Group1	FTAI	8.4 (4 - 12)	4.3 (1 - 8)	37 (12 - 74)	228.3 (182- 296)	245.4 (200 - 308)	3.4 (3 - 4)	3.8 (3 -4)
	NM	8.6 (4 – 14)	4.6 (1 -10)	66 (35-104)	221.2 (167 -315)	242.4 (200 - 308)	3.4 (3 – 4)	3.7 (3-4)
Group2	FTAI	6.6 (4 – 14)	2.8 (1 -10)	58 (10-112)	240.7 (200 –282)	252.2 (214 – 287)	3.6 (3 – 4)	3.8 (3–4)
	NM	6.4 (4 – 9)	2.8 (1 – 5)	63 (4 –108)	236.9 (204 –273)	250.4 (216 – 287)	3.6 (3 – 4)	3.7 (3–4)
Group3	FTAI	7.7 (4 – 13)	3.8 (1 – 9)	96 (32-504)	215.2 (159 –285)	238.6 (177 – 304)	3.2 (3 – 4)	3.7 (3–4)
	NM	7.5 (4 – 13)	3.5 (1 – 9)	92 (34-124)	217.6 (175 –275)	242.8 (191 – 298)	3.3 (3 – 4)	3.7 (3–4)
Group4	FTAI	7.3 (4 – 13)	3.3 (0 -10)	123 (32-805)	221.8 (174 -308)	246.6 (190 – 336)	3.4 (3 – 4)	3.8 (3–4)
	NM	6.8 (4 – 13)	1.5 (1 – 9)	145 (32-1177)	232.3 (175 –312)	250.7 (186 – 330)	3.5 (3 – 4)	3.7 (3–4)

Table 14 shows the descriptive statistics of the four groups used to compare the effect of NM against FTAI during the mating season.

The oestrus behaviour observed in the treated cattle are summarised in Table 15. There were significant differences between groups in the proportion of cattle which showed behavioural signs (X^2=25.2, p<0.001).

Table 15 Oestrus behavioural signs for cows treated with PGF2α in the four groups.

Group No	Stand to be mounted	Mount others	Mucus discharge
1	3	3	3
2	6	6	6
3	1	1	1
4	0	0	0

The results of the pregnancy test on 26th and 27th of June, 2016 are summarised across group and treatment method in Table 16.

Table 16 Summary of pregnancy results across group and treatment method.

Group No	Treatment groups	No. of pregnant*
1	NM	10
	FTAI	11
2	NM	14
	FTAI	10
3	NM	14
	FTAI	12
4	NM	11
	FTAI	6
TOTAL	NM	49/100
	FTAI	39/100

*, 50 animals in each group, with 25 per treatment group

At the end of the project, 39/100 cows were pregnant in the FTAI group and 49/100 cows were pregnant in the NM group. The RR of pregnancy of the FTAI group against that of the NM group with 95% CI was 0.8; 95% - CI 0.58-1.09.

The results of the univariable logistic regressions are summarised in Table 17. Of the factors tested only three, days from last calving, breeding method and Bodyweight one month prior to start of breeding season had any association with the odds of pregnancy (all P<0.25).

Table 17 Results of univariable logistic regression with their respective odd ratios and p-values.

<table>
<tr><td>Explanatory variable</td><td colspan="2">Odds ratio (95% CI)</td><td>P-value</td></tr>
<tr><td>Age</td><td colspan="2">1.009 (0.912 – 1.116)</td><td>0.87</td></tr>
<tr><td rowspan="2">Parity (categorical)
Reference: parity 5+</td><td>Parity 0-1</td><td>0.89 (0.38 – 2.1)</td><td rowspan="2">0.72</td></tr>
<tr><td>Parity 2-4</td><td>1.19 (0.61 – 2.3)</td></tr>
<tr><td>Days from last calving to start of study</td><td colspan="2">0.998 (0.995 – 1.001)</td><td>0.18</td></tr>
<tr><td>Breeding method</td><td colspan="2">0.696 (0.397 – 1.221)</td><td>0.21</td></tr>
<tr><td>Body weight one month prior to start of breeding season</td><td colspan="2">1.006 (0.996 – 1.015)</td><td>0.25</td></tr>
<tr><td>Body weight at start of breeding season</td><td colspan="2">1.005 (0.995 – 1.015)</td><td>0.30</td></tr>
<tr><td>BCS one month prior to start of breeding season</td><td colspan="2">0.967 (0.549 – 1.700)</td><td>0.91</td></tr>
<tr><td>BCS one month at start of breeding season</td><td colspan="2">1.371 (0.728 – 2.582)</td><td>0.33</td></tr>
<tr><td rowspan="3">Group No.
Reference: Group 1</td><td>Group 2:</td><td>1.4 (0.63 – 3.1)</td><td rowspan="3">0.35</td></tr>
<tr><td>Group 3:</td><td>1.5 (0.68 – 3.3)</td></tr>
<tr><td>Group 4:</td><td>0.78 (0.34 – 1.7)</td></tr>
<tr><td>Change in weight pre-breeding</td><td colspan="2">0.996 (0.973 – 1.019)</td><td>0.73</td></tr>
<tr><td>Change in BCS</td><td colspan="2">0.707 (0.381 – 1.310)</td><td>0.27</td></tr>
</table>

These three factors (as well as their interactions with breeding method) as shown in Table 17 were then included in the multivariable logistic regression. In the final model, only breeding method (which had been forced into the model) remained. In this final model, the odds ratio for pregnancy in cattle given an FTAI, compare to those which were naturally mated was 0.73 (95%CI 0.4-1.24; p= 0.224).

Chapter 5

Discussion

The aim of this project was to assess whether using a $PGF_{2}\alpha$ synchronization protocol in Mpwapwa cattle would improve reproductive performance. A standard 14-day $PGF_{2}\alpha$ synchronization protocol with FTAI was compared to NM over a 12-week breeding season. At the end of the project, 39/100 cows were pregnant in the FTAI group and 49/100 cows were pregnant in the NM group. Although there was no significant difference in pregnancy rate between the two breeding groups (P=0.21), the RR of pregnancy in the FTAI group was appreciably lower than that of the NM group (unadjusted RR=0.8; 95% - CI 0.58-1.09). However, cattle in the FTAI-treated group were only inseminated once, whereas the NM group could be naturally mated on multiple occasions during the 12-week breeding season; In addition, the use of $PGF_{2}\alpha$ synchronization allowed the use of AI which is not feasible under most Tanzanian systems when cows come into oestrus naturally. Thus, the results of this study suggest that $PGF_{2}\alpha$ -based synchronization, particularly if used alongside natural mating, can improve the reproductive performance of Mpwapwa breed cattle as well as allowing for greater genetic gain than occurs with natural mating.

Prostaglandin $F_{2}\alpha$ and breeding methods

The proportion of cows that came into heat and displayed behavioural signs after administration of the first $PGF_{2}\alpha$ injection was very low (only 10/100 cattle). This proportion was much lower than expected: Hafs et al. (1975) reported that only 10/33 suckling beef cattle failed to show oestrus after an injection of cloprostenol, while Landivar et al. (1984) reported that 46% of Zebu cattle with a palpable CL showed oestrus after a first injection of $PGF_{2}\alpha$ (equivalent to an overall rate of ~25% if all cattle had been treated irrespective of CL status).

The reason for this poor response is unclear. It could be that oestrus detection was not very effective, or that a higher than expected proportion of cattle did not have a responsive CL. The most likely cause of the later is a higher proportion of cattle in anoestrus. $PGF_{2}\alpha$ is ineffective at synchronizing cows in anoestrus, even when used in a double injection program (i.e. because it relies upon the presence of a responsive CL), so if anoestrus was present it would have reduced the conception rate to the FTAI.

Further investigation of the reproductive state of Mpwapwa cattle at the start of the breeding season might identify whether anoestrus is a significant cause of poor reproductive performance. If anoestrus is common, identifying cattle in anoestrus at the start of the season could be useful, as they could be treated using progesterone-based programmes (McDougall, 2010) and cattle with a CL could be treated with $PGF_{2}\alpha$. However, accurate identification of reproductive status requires skilled veterinarians or technicians and may not be feasible in most situations. If this is the case and anoestrus is a problem, then blanket treatment of cattle (or selected group of cattle) with progesterone-based programmes as is practiced in New Zealand (McDougall, 2010) may be more feasible and be more effective.

Other factors affecting fertility in beef cattle

Bulls

The fertility of the bull plays an important role at the herd level when there is natural mating, but is also significant when AI is used. In most cases, bulls used for natural mating on Tanzanian farms are not tested prior to be being used for breeding. It is recommended by Parkinson (2004) that, a breeding soundness examination (BSE) should be conducted at least two months prior to the start of the mating season in order to ensure the right bulls are used for the breeding. Scrotal circumference (SC), mating ability, and semen quality testing are the most important components of a BSE. Libido, social dominance, and service capacity are the not normally evaluated during BSE do have a positive effect on bull's fertility on cow herd. The four bulls that were used to naturally mate cows in the four batches of the control group were aged ≥ 3 years and had SC ≥30cm, good mating ability and better conditions in terms of live body weight ≥240kg at pre-breeding weight and ≥235kg at post-breeding weight (Table 11). McGowan et al. (2002) reported that the majority of the bulls have attained puberty once their SC is ≥ 30 cm, also there is a direct relationship between the size of the testes and sperm production. The gross motility of semen for the semen straws collected from the two Mpwapwa breed bulls at the National Artificial Insemination Centre used for the present study was ≥60% (Table 12). Based upon the methods used for performing BSE in this study, it is recommended that programmes for BSE should be introduced into Tanzanian farms for evaluation of bulls to be used during the breeding season, with culling of unsatisfactory ones.

Management of animals

Body condition score

The cows in this study underwent a one-month programme of nutrition and preventative medicine treatments programme in order to optimise BCS prior to the start of breeding season. All cows were either BCS 3 (56.5%) or 4 (43.5%) prior to the programme. As BCS of 3 is deemed unsatisfactory for the start of the breeding season, this means that over 50% of cattle of the 200 selected cows were not in good condition after finishing the dry season. However, by the end of the pre-treatment month, the proportion of cows at BCS 4 cows had increased to 72%. (Table 13). On the other hand, there was no significant difference in pregnancy rate between BCS 3 and 4, indeed the relative risk of pregnancy on cows with BCS 4 was lower (unadjusted RR=0.6; - CI 0.46-0.69) than that of cows with BCS 3 (unadjusted RR=1.8; - CI 1.46-2.16). This result was unexpected, in as much as, published data strongly suggest that BCS is an important component of fertility (Rae et al., 1993). Nonetheless, despite the lack of a difference in pregnancy rate in the present study, programmes for checking cows' BCS should probably be introduced in Tanzanian farms to detect and monitor cows in poor conditions prior to the start of the breeding season. It should be noted that, even though there was no difference in pregnancy rate between cows in BCS 3 and BCS 4, all cows had been well-fed for the month before the start of mating. Whether this would have been the case if the animals' plane of nutrition had not been improved would be a matter for further investigation.

Pre-pre-breeding weights and pre-breeding weights

The pre-breeding programmes significantly improved body weight (Table 14); in particular, except in the last batch of cattle, all cows had a bodyweight >200kg. The recommended minimum breeding weight for Mpwapwa cattle is 180kg (which all cattle had attained at the point of selection into the study), although there are no published data to support this figure. Further research is required to better establish optimal weights for breeding Mpwapwa cattle and to identify whether there is any advantage to weighing in addition to assessing BCS.

Conclusion

Despite a relatively intensive pre-breeding programme and the use of tested bulls, at the end of the breeding season only 49/100 of NM cattle were pregnant. The percentage of cattle in the FTAI group was lower (39/100), but these cattle were only inseminated once and they became pregnant at the beginning of the breeding season, in contrast to the control group which would have got pregnant over a long period of time. Furthermore, all 39 cattle were pregnant to AI, so their calves were of significantly better genetic merit than those of the control group. This pilot study has therefore shown that a simple 14-day prostaglandin protocol can produce pregnancy rate from a single insemination almost as good as those resulting from breeding throughout the season, while at the same time facilitating the use of AI.

The low pregnancy rates in the control group and the low proportion of the treated cattle showing oestrus strongly suggest that there are underlying problems with the cyclicity of the Mpwapwa cattle selected for this study. Further research should look at the follicular dynamics of Mpwapwa cattle at the start of the breeding season, to identify whether the issues are at the herd level or whether there is collection of individual cow problems. Such research could identify the optimal synchronization programme to use in these cattle, either replacing the prostaglandin programme or to be used alongside it. Based on the extensive available literature, the use of FTAI implies no oestrus detection at all.

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