



Response to hormonal treatment and conception rates of Sahiwal cows subjected to fixed time artificial insemination in pastoral dairy systems

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Abstract

This study aimed at determining factors influencing response of Sahiwal cows/heifers to fixed time artificial insemination protocol in pastoral systems in Kenya. Available cows/heifers were inspected for conformity to Sahiwal breed characteristics, parity, body condition score, and subsequently rectal palpation to determine pregnancy status, ovarian structures, and estimated ovarian diameter. Consequently, these animals were injected with 100 µg of gonadotrophin-releasing hormone. On days 7 and 9, only responsive cows/heifers were injected with 500 µg of *cloprostenol* and 100 µg of *gonadorelin Acetate*, respectively. On day 10, animals were inseminated and separated from bulls for 45 days and pregnancy diagnosis done after 90 days. Analysis of variance was performed to determine the effects of production system, parity, and ovarian structures on ovary diameters pre- and post-hormonal treatment. Logistic regression was used fitting a logit function to account for the binomial distribution of conception. Overall, 56.2%, 23.1%, and 20.7% of the animals had follicles (F), corpus luteum (CL), and corpus albicans (CA), respectively, at day 0, and 16.6%, 68.6%, and 14.8%, respectively, at day 7. Human and environmental factors had no influence on conception. Among the animal factors, only the ovarian structures at day 7 had a significant effect on conception. Ovaries with CL at this time were about 6 times significantly more likely to conceive than those with F. For higher conception rates, animals with ovaries with CL should be recruited into the FTAI program as they are significantly more likely to conceive than those with other ovarian structures.

Keywords Fixed-time artificial insemination · Reproductive technologies · Pastoral dairy systems · Estrus synchronization · Sahiwal cattle

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Introduction

Pastoral dairy cattle systems provide for almost 80% needs of the milk requirements of the arid and semi-arid areas (ASALs) that comprise over 80% of the Kenyan land mass (Kahi et al., 2004; Ojango et al., 2016). Milk production in these areas are mainly from indigenous cattle (East African Zebu), dual purpose cattle (Sahiwal), and their crossbred genotypes with the *Bos taurus* (KNBS, 2019). The Sahiwal is preferred by pastoralists to other zebu breeds due to its high milk production, growth performance, good reproductive ability (Ilatsia et al., 2007), and their relative adaptation to the challenging range conditions (Ilatsia et al., 2011). Despite the impressive results from this breed and subsequent high demand for milk and beef due to increase in population, supply of superior bulls and cows from the nucleus herd to the commercial herds remain low (Mwangi & Ilatsia 2021). Improving reproductive efficiency of the

breed has been identified as one of measures that may be exploited to alleviate the problem.

Reproductive performance directly impacts on onset of milk production of cows and consequently the production herd life of the cow and the total lifetime milk production (Ojango et al., 2016). It also influences rate of replacement and the selection intensity in the herds since it affects the number of available animals for selection. Primarily, pastoral dairy systems utilize natural mating to serve their cows (Awulachew, 2021). Bulls are allowed to roam within female herds, mating with any female that show signs of Estrus. Consequently, cows calve down throughout the year. This possess management challenges especially for calves born during the dry season. In order to enhance conception, more than one bull are allowed in the herd. In such cases, it is not possible to determine the paternity of the calves born in these herds. This results into incomplete pedigrees that have negative impacts on breed improvement efforts (Wasike et al., 2011; Nilforooshan et al., 2021). In addition, keeping bulls in the herds increases the cost of herd management which could otherwise be saved if use of assisted reproductive technologies (ART) was adopted (Lardner et al., 2020).

Assisted reproductive technologies have been existence since the early twentieth century (Parish and Riley, 2011). Some of these technologies include artificial insemination (AI), oestrus synchronization (ES), semen sexing, multiple ovulation and embryo transfer (MOET), invitro fertilization (IvF), and their combinations (Daly et al., 2020; Lardner et al., 2020). The extent of use of these technologies vary with AI leading in popularity. Though AI is extensively used in specialized commercial dairy systems, its utilization remains low in pastoral systems where indigenous livestock are predominant and livestock play multiple roles (Kosgey et al., 2011; Lardner et al., 2020). The deterrence to utilization of AI in pastoral systems arises from cost, environmental, and infrastructural challenges. As a result, use of ART especially FTAI has been introduced and promoted in pastoral dairy systems to enable use of superior genetic resources especially Sahiwal cattle as a strategy towards breed improvement to enable increase in milk production.

Fixed time artificial insemination involves hormonal treatment of the cows/heifers to synchronize their oestrus cycles so that they all come on heat at relatively same time. The cows/heifers that manifest oestrus are then artificially inseminated using frozen semen. There are various protocols that have been advanced for FTAI such as 3-day, 7-day, 10-day, and 14-day protocols with varied procedures of hormonal treatment of animals (Lamb et al., 2006; Nowicki et al., 2017). Equally, the resultant conceptions from these protocols in different studies remain inconsistent. Therefore, this study aimed at determining the factors that influence response of Sahiwal cows/heifers to hormonal treatment and the success rates of insemination under FTAI in pastoral dairy systems.

Materials and methods

Study area

The study was carried out in Kajiado and Narok counties from March to August 2021. Kajiado County represented an extensive pastoral production system, while Narok County represented a semi intensive pastoral production system. Kajiado County lies between $-1^{\circ} 51' 8.57''$ S and $36^{\circ} 46' 36.59''$ E at altitudes between 500 and 2500 m above sea level. The county covers an area of 2,1292.7 km². The region has four ecological zones: semi-humid to semi-arid, semi-arid, arid, and very arid. It is occasioned by seasonal rains that fall in two seasons, the long rains season fall in March to May, and the short rains are from October to December. The mean annual rainfall ranges from 300 to 800 mm. Pastoralism is the main source of livelihood for majority of the households. Livestock are grazed in communally managed or open access natural pastures. During dry seasons, livestock are migrated to other areas such as Amboseli and even in the neighboring Tanzania (County Government of Kajiado, 2019).

Narok County lies between $1^{\circ} 5' 15.1548''$ S, $35^{\circ} 52' 37.4304''$ E, and the altitude peaks at 3100 m above sea level. The county covers an area of 17,933.1 km². The county has 4 agroclimatic zones, namely, humid, sub humid, semi humid, to arid and semi-arid (Suke, 2020). Two-thirds of the county are classified as semi-arid. The county has bimodal rainfall with long rains between February and June and short rains between August and November. The mean rainfall ranges from 500 to 2500 mm. Data was collected at Keiyian cooperative farm, which is located in Trans-Mara West Sub County. The altitude ranges between 900 to 1950 m above sea level with a mean annual rainfall between 1000 and 1800 mm. The rainfall is bimodal with long rains from March to May and short rains in August to November and mean temperature ranges from 14.8 to 20.3 °C (Suke, 2020).

Synchronization protocol and data collection

Animals were assembled into five insemination camps in Kajiado county distributed in three Sub-counties, namely, Kajiado Central, Mashuru, and Kajiado North. There were two camps in Kajiado central, one camp in Mashuru and two camps in Kajiado North. Narok had only one camp. The Sahiwal cows/heifers used in the study were inspected for conformity to Sahiwal breed characteristics as described by Muhuyi et al. (1999), parity and body condition score. Cows beyond 3rd parity and body score below 2.5 were excluded and thus exited the camp. The cows/heifers that passed this first stage of examination proceeded to the second stage where they were examined by rectal palpation to determine

pregnancy status, ovarian structures, and estimated ovarian diameter. The ovarian structures were used to determine the cyclicity of the selected cows/heifers. Key structures palpated were follicles (F), corpus luteum (CL), and corpus albicans (CA).

A 10-day OvSynch protocol was used for estrus synchronization and AI. At the start of estrus synchronization protocol (day zero), the selected animals were injected with 100 µg of gonadotrophin releasing hormone (GnRH) analogue *gonadorelin Acetate* intramuscularly on the neck muscles. The time of injection for the first and last animal injected was then recorded. On day 7, the cows/heifer recruited on day 0 were examined by rectal palpation to confirm their non-pregnancy status and their response to the hormonal treatment based on the changes in ovarian structures (follicles, corpus luteum, and corpus albicans). All non-pregnant and responsive cows/heifers were injected with 500 µg of *cloprostenol* intramuscularly on the neck same as day 0 injection time. On day 9, all animals that were treated on day 7 were injected with 100 µg of *gonadorelin acetate* at the same injection time as on day 7. On day 10, animals treated on day nine were inseminated between 14 and 16 h after the last *gonadorelin acetate* injection. A schematic presentation of the protocol is shown in Fig. 1.

At the time of insemination, the identity of sire used and the inseminator were recorded. The inseminated animals continued to stay separated from the bulls for 45 more days. Pregnancy diagnosis by rectal palpation was done 90 days post-insemination.

Data preparation and analysis

The data was divided into two sets. Data set 1 included 168 animals that were selected and treated with hormones from day 0 to day 7. Data set 2 included 79 animals that went through FTAI and were pregnancy diagnosed after 90 days. Normality tests of the ovarian diameter data using the

Shapiro–Wilk test showed normal distribution ($p < 0.001$). Analysis of variance using the PROC GLM of SAS (SAS v. 9.1, 2002) was performed to determine the effects of production system, parity, and ovarian structures on ovary diameters pre- and post-hormonal treatment. To assess animal response to GnRH, change in ovarian structures pre- (day 0) and post- (day 7) hormonal treatment were determined. Given that ovarian structure data was categorical, this was converted to count data, and thereafter, a chi-square test was used. In both analyses, data set 1 was used.

To determine factors influencing conception, a logistic model presented in Eq. 1 was fitted on data set 2. The factors included animal (parity, ovary diameter at day 0, ovarian structures at day 0, ovary diameter at day 7, and ovarian structures at day 7), environmental (production systems), and human (inseminator and time of insemination) effects. The PROC LOGISTIC function of SAS (SAS v. 9.2, 2008) was used for the analysis, and a logit function fitted to account for the binomial distribution of the response variable.

$$Y_{ijklmn} = P_i + OS_j + T_k + PS_l + I_m + \epsilon_{ijklmn}$$

where y_{ijklmn} is the conception status (positive or negative) of animal n ; P_i is the effect of i th parity ($i=0, 1, 2$); OS_j is the effect of j th ovarian structure (j =follicle, corpus luteum, corpus albicans); T_k is the effect of k th insemination duration ($k=1, 2, \dots, 7$); PS_l is the effect of l th production system (l =extensive, semi-intensive); I_m is the effect of m th inseminator ($m=1, 2, 0.4$); ϵ_{ijklmn} is the random error term (assumed to be independent, normally distributed with zero mean and constant variance).

Time to insemination for each individual was computed as the difference between time of insemination on day 10 and time of GnRH injection on day 9. This resulted in a range of 13 to 15 h across the study population. Time to insemination (13 to 15 h) was categorized into three groups from time 1 (T1) to time 3 (T3) with each group differing by 40 min.

Fig. 1 A schematic presentation of 10-day OvSynch protocol

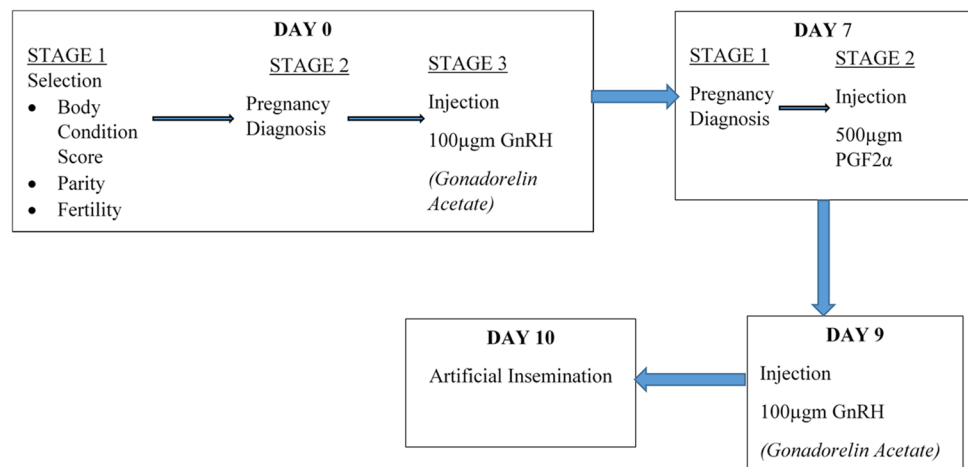


Table 1 Descriptive statistics of number of animals subjected to FTAI and their associated percentages in parentheses

Parameters	Production system		Overall
	Extensive	Semi-intensive	
Animals inseminated	129 (76.3)	40 (23.7)	169 (100)
Ovarian structures of animals inseminated at day 0			
Follicle	78 (82.1)	17 (21.9)	95 (100)
Corpus luteum	30 (76.9)	9 (23.1)	39 (100)
Corpus albicans	21 (60.0)	14 (40.0)	35 (100)
Ovarian structures of animals inseminated at day 7			
Follicle	19 (67.9)	9 (32.1)	28 (100)
Corpus luteum	95 (81.9)	21 (18.1)	116 (100)
Corpus albicans	15 (60.0)	10 (40.0)	25 (100)
Number of animals' pregnancy diagnosed post-FTAI	40 (50.6)	39 (49.4)	79 (100)
Number of animals confirmed pregnant	24 (51.1)	23 (48.9)	47 (100)

Results

The number of animals, their ovarian structures, and pregnancy status are presented in Table 1. A total of 169 animals qualified for FTAI out of which 76.3% were in extensive and 23.7% in semi-intensive system. On the overall, 56.2%, 23.1%, and 20.7% of the animals had follicles, corpus luteum, and corpus albicans, respectively, at day 0, and 16.6%, 68.6%, and 14.8%, respectively, at day 7. At day 0, animals with follicles, corpus luteum, and corpus albicans in the extensive system were 60.5%, 23.3%, and 16.3% of, respectively, while in the semi-intensive system, they were 42.5%, 22.5%, and 35.0%, respectively. At day 7, however, 14.7%, 73.6%, and 11.6% of animals had follicles, corpus luteum, and corpus albicans, respectively, in the extensive system, and 22.5%, 52.5%, and 25.0%, respectively, in the semi-intensive system. Whereas only 30.0% of animals in the extensive system were available for post FTAI pregnancy diagnosis, 97.5% were available in the semi-intensive system. Out of the available animals, the conception rates were 60.0% and 59.0% in extensive and semi-intensive systems, respectively.

The factors influencing ovarian diameter at days 0 and 7 are presented in Table 2. The overall mean diameter at day 0 and 7 were 1.6 ± 0.07 cm and 1.7 ± 0.08 cm, respectively. Production system and parity had no influence on ovary diameter at both days. At day 0, ovaries with follicles (F) and corpus luteum (CL) had similar diameter that was significantly higher than for ovaries with corpus albicans (CA). At day 7, ovaries with CL had significantly higher diameter than both ovaries with F and CA.

The effect of GnRH treatment on ovarian structures pre- and post-treatment is presented in Table 3. There was a significant ($P < 0.05$) response in the proportion of ovarian structures between day 0 and day 7. The proportion of follicles (F) reduced by about 40%, corpus luteum (CL)

Table 2 Factors influencing ovary diameter at days 0 and 7

Effect	Ovary diameter at day 0 (cm) ⁱ	Ovary diameter at day 7 (cm)
Production system	ns	ns
Extensive	1.5 ± 0.14	1.4 ± 0.15
Semi-intensive	1.7 ± 0.09	1.5 ± 0.10
Parity	ns	ns
Heifers	1.5 ± 0.08	1.5 ± 0.08
1st Calvers	1.8 ± 0.16	1.6 ± 0.17
2nd Calvers	1.5 ± 0.22	1.3 ± 0.22
Ovarian structures	***	***
Follicles	$1.8 \pm 0.12a$	$1.3 \pm 0.17b$
Corpus Luteum	$2.0 \pm 0.15a$	$2.1 \pm 0.11a$
Corpus Albicans	$1.0 \pm 0.12b$	$1.0 \pm 0.13b$
Overall mean	1.6 ± 0.07	1.7 ± 0.08

ⁱWithin an effect, means in a column followed by different letters are significantly ($P < 0.05$) different; ns = not significantly ($P \geq 0.05$) different; *** significantly different at 0.001

Table 3 Ovarian structure response to gonadotrophic releasing hormone (GnRH) pre- (day 0) and post- (day 7) treatment

Ovarian structure	Day 0	Day 7	Chi-square	p-value
Follicle (%)	56.2	16.6	76.41	<0.001
Corpus luteum (%)	23.1	68.6		
Corpus albicans (%)	20.7	14.8		

increased by about 46%, while corpus albicans reduced by about 6%.

Animal, environmental, and human factors influencing conception are presented in Table 4. Human (inseminator and insemination time) and environmental (production

system) factors had no influence on conception. Among the animal factors (parity, ovarian structures at day 0 and ovarian structures at day 7), only the ovarian structures at day 7 had a significant effect on conception. Ovaries with CL at this period were about 6 times significantly more likely to conceive than those with F.

Discussion

Artificial insemination (AI) is a widely used reproductive technology for dissemination of superior genetics in the high rainfall dairy zones of Kenya (Ojango et al., 2016). In the semi-arid pastoral areas, however, adoption of the technology is relatively low due to several challenges that include accessibility, affordability, estrus detection efficiency, and availability of skilled technician (Mwai et al., 2020). The application of estrus synchronization (ES) and fixed time artificial insemination (FTAI) technology is possible to increase adoption of AI in such areas. The fact that more than 169 cows/heifers were willingly availed for the study may be an indicator of the awareness and willingness of the pastoralists to adopt AI. Indeed, the demand for Sahiwal genetics in the study area has been reported to exceed the annual availability of breeding bulls from the national Sahiwal stud (Mwangi and Ilatsia, 2021).

On the overall and in both production systems, the follicle (F) was the predominant ovarian structure at day 0. Although an on-line literature search did not yield any similar study for comparative purposes, the finding in the current study was in close agreement with the results of Mahzabin et al. (2020). In that study, 62.5% of ovaries collected from an abattoir were in the follicular stage and had no CL, while the rest (37.5%) had CL. The estrus cycle is hormonally controlled and divided into estrus, metestrus, diestrus, and proestrus phases (Noseir, 2003). The proestrus and estrus are termed as the follicular phase. At the proestrus phase, pre-existing primordial follicles develop in wave-like patterns into ovarian follicles, with generally one follicle becoming dominant (Webb et al., 2016). At estrus, the dominant follicle is ovulated in readiness for fertilization. Concurrently and in preparation for fertilization, metestrus and diestrus (known as the luteal phase) are initiated. Metestrus, the early stage of CL formation is characterized by presence of corpus hemorrhagicum (CH), while diestrus is characterized by a prominent functional CL (Maina et al., 2008). If fertilization fails after ovulation, the CL regresses into a corpus albicans (CA). The presence of these structures in the current study therefore indicate that the animals were cycling and that most were in the follicular phase. The ratios of the structures were not significantly different between production systems (figure not shown) indicating that the conditions in both systems were similar.

Table 4 Animal, environmental, and human factors influencing conception in animals subjected to fixed time artificial insemination (FTAI)

Factor	Effect	df	Effect level ⁱ	Odds ratio	P-value ^j	95% CI
Animal	Parity	2	1	2.14	0.40	0.36–12.74
			2	3.98	0.23	0.42–37.57
			0	1	Ref	Ref
	Ovarian structure at day 0	2	CL	2.39	0.21	0.62–9.24
			CA	1.57	0.54	0.38–6.48
			F	1	Ref	
	Ovarian structure at day 7	2	CL	6.39	0.02*	1.38–29.62
			CA	2.94	0.22	0.53–16.35
			F	1	Ref	
Environment	Production system		Semi-intensive	0.12	0.23	0.01–3.40
			Extensive	1	Ref	
Human	Inseminator	3	2	1.22	0.80	0.26–5.68
			3	7.91	0.22	0.29–217.17
			4	0.72	0.87	0.01–36.74
			1	1	Ref	
	Time to insemination	1	T2	0.91	0.88	0.27–3.03
			T3	0.72	0.70	0.13–3.88
			T1	1	Ref	

ⁱF follicle, CL corpus luteum, CA corpus albicans, T1 to T3 time from the last hormonal treatment to insemination

^jRef = reference class

*Significant at 0.05

Fewer animals were available for pregnancy diagnosis (30%) in the extensive system than in the semi-intensive system (97.5%). This was due to livestock migration in search of pastures due to onset of drought. Although the Maasai community are sedentary with permanent settlements, seasonal livestock migration occurs from permanent settlements to temporal grazing areas. A study by Butt et al. (2009) suggested that household relocation reduces the stress faced by pastoralists and their cattle during the drought. Nevertheless, the conception rates observed in this study (60.0% and 59.0% in extensive and semi-intensive systems, respectively) were within the range reported for *Bos indicus* beef breeds in Argentina (Bó & Baruselli, 2014), irrespective of the protocol used. The results are also consistent with conception rate of 58% in a *Bos taurus* breed reported by Lardner et al. (2020). It may therefore be inferred that the protocol used led to acceptable conception rates.

The overall estimated ovarian diameter at day 0 and day 7 (1.6 and 1.7 cm, respectively) observed in this study are within the range of 1.5 to 1.9 cm reported by various authors in studies employing actual measurements of ovaries collected from slaughter houses (Kunbhar et al., 2003; Kouamo et al., 2014; Islam et al., 2018; Ali et al., 2021). The results by Mahzabin et al. (2020), however, reported lower diameter (1.1 and 1.2 cm) than in the current study. This may be attributed to the cyclicity status of the cows/heifers. Various studies have also shown that the nutritional status, the breed, the presence of follicles, and CL influence the size of the ovary (Cushman et al., 2014; Peralta-Torres et al., 2017). Lack of significant differences in ovarian diameter at days 0 and 7 between production systems and parities is a confirmation that the cows/heifers in the extensive and semi-intensive systems as well as in the different parities were in a similar state of normal estrus cycle (Sharma et al., 2018). The significantly lower diameter of ovaries having CA as the dominant structure than for ovaries with both F and CL as the dominant structures at both day 0 and 7 was in agreement with 1.1 and 1.2 cm reported by Mahzabin et al. (2020). These authors explained that the ovaries were obtained from animals that had been culled due to their low reproductive performance. The scenario in the current study could be associated with animals most likely being at the proestrus phase of the estrus cycle. The significantly wider ovary diameter containing CL as the dominant structure at day 7 agrees with Kouamo et al. (2014) and Ali et al. (2021) who reported significantly wider CL present ovaries than those without.

The significant increase in ovarian diameter at day 7 of the ovaries that had CL than both F and CA and the significant increase in the number of animals (46%) that had CL at day 7 (Table 3) is a clear indication of response to GnRH treatment that was carried out at day 0. Administration of GnRH for animals with F induced release

of luteinizing hormone (LH) leading to ovulation of the dominant follicle and emergence of a new follicular wave about 1.5 to 2 days later (Bó & Baruselli, 2014). Additionally, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) surge leads to ovarian function, such as follicular growth and maturation, ovulation, corpus luteum formation, and maintenance (Kim, 2018). Hence, some cows/heifers that had CL at day 0 persisted to day 7 after being treated with GnRH while those animals that had CA, the GnRH caused a CL to be formed. This resulted in numbers of animals that had CL to increase by about 46%, while those with F and CA reduced by about 40 and 6%, respectively.

Prostaglandin $F_{2\alpha}$ and its analogues are luteolytic in cattle and are effective on CL from days 5 to 17 of the estrous cycle. Injection of cloprostenol on day 7 leads to the regression of the CL, and hence, the progesterone effect on the reproductive system is reduced leading to production of LH. Treatment with GnRH on day 9 induces release of LH and ovulation of the dominant follicle (Bó & Baruselli, 2014). In this study, conception rate was 60% indicating that 40% of animals that had F and CL at day 7 did not conceive. This is because $PGF_{2\alpha}$ hormone administered on day 7 sustains the follicle, and hence, primordial follicles would endure after administration of GnRH on day 9. Additionally, primordial follicles (non-growing follicles) and growing (primary, secondary, and tertiary) follicles have been found in the ovary undergoing either development or degeneration as a wave-like pattern (Bihon and Assefa, 2021). This implies that follicles are continuously in processes of recruitment, dominance, selection, and atresia where some follicles are entering from the non-growth phase to a growing population. As a result, these animals with primordial follicles on day 9 would not conceive during insemination because of their follicles not being mature. This means that only animals that had follicles in growth phase conceived. This is in agreement with a study by de Lima et al. (2020) that showed that the pregnancy rate was higher in animals with low antral follicle count than in those with a high antral follicle count.

Successful synchronization of ovulation relies on the stage of follicular development at the time of initial GnRH treatment at day 0. For higher conception rates animals with ovaries with CL should be recruited into the FTAI program as they are significantly more likely to conceive than those with other ovarian structures.

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Author contribution All authors contributed to conception and design of this study. Material preparation, data collection, and analysis were performed by Mwaura Magothe, Daniel Mwangi, Bwire Wasike, Ruth Waaineina, Sophie Miyumo, Samson Mwangi, and Evans Ilatsia. The first draft of the manuscript was joint effort by all authors who also

commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are not publicly available due to the lead institution (KALRO) policies but are available from the corresponding author on reasonable request.

Declarations

Ethical approval The study was exempted from requiring ethical approval since the data used was from a community engagement project.

Competing interests The authors declare no competing interests.

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