*Original Research Article*

Assessing the Feasibility of using Ambient Temperature Semen and Prostaglandin-Based Synchronisation in Beef Cattle under Tropical Conditions of Tanzania

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ABSTRACT

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| Artificial insemination (AI) services for the Mpwapwa breed of cattle in Tanzania have been in abeyance for many years, largely due to difficulties of supply of liquid nitrogen to remote and rural areas. Historically, AI using unfrozen ambient temperature or chilled semen have been effective alternatives to cryopreserved (frozen) semen, subsequently, AI using ambient temperature semen could form the basis of an AI service for the Mpwapwa region (and other similar areas across East Africa). However, the feasibility of using ambient temperature semen needs testing in field conditions. In this study 151 Mpwapwa cows were synchronised using a standard 14-day double prostaglandin (PG) programme and inseminated using ambient temperature semen (AT). Cows showing oestrus after the first PG injection were inseminated accordingly; all other cows were inseminated at ~56 hours after second PG. Bulls were then run with the cows for 60 days, after which conception rates to AI and overall pregnancy rates were determined by transrectal ultrasonography. Conception rates to AI with AT semen were 62% (94/151), while final pregnancy rates (after natural mating) were 96% (145/151). In this study, the conception rate to AI with ambient temperature semen was at least as good as that which has been achieved with frozen semen under similar conditions, and costs were at least half of the costs to create and use frozen semen, demonstrating the potential of AT semen to be used in rural Tanzania. |

*Keywords:* Ambient temperature semen, Artificial insemination, Oestrus synchronization, Pregnancy rates

1. INTRODUCTION

The Mpwapwa breed of cattle was developed in the 1950s as a means of improving the quality of livestock available to cattle farmers in Tanzania. Initially very successful [1], the development and dissemination of the Mpwapwa breed was interrupted by the suspension of nation-wide cattle AI services in the 1970s. A nucleus herd was maintained at the Tanzania Livestock Research Institute (TALIRI) in Mpwapwa. This herd was subjected to a degree of genetic improvement and selection, but dissemination of the breed was largely limited to supplying small numbers of bulls to individual farmers. Breeding beef cattle on-farm by artificial insemination (AI), including the Mpwapwa breed, became extremely limited, and has had generally poor results [2,3,4]. Recently, however, there has been renewed interest in breeding Tanzanian beef cattle by AI and to that end, the TALIRI Mpwapwa facility has been re-equipped for undertaking AI [5].

One of the most difficult problems to overcome during the redevelopment of an AI programme based at TALIRI Mpwapwa has been reliably obtaining liquid nitrogen (LN2) for the production and maintenance of cryopreserved semen. Cryopreserved semen not only requires substantial quantities of LN2 at the production/storage centre, but it also requires that LN2 is available and transportable to the point of insemination. Whilst this is feasible to some extent in the higher density cattle populations of the dairy-farming areas of the country, LN2 availability in the beef-rearing areas presents a significant limitation to the restoration of an AI service. As a result, preservation of frozen semen becomes not only a key challenge in the TALIRI Mpwapwa breeding programme, but also to smallholder farmers using AI services in the Mpwapwa district.

A potential solution to this problem is to use an alternative method of semen extension and preservation. When AI programmes were first developed, they were based upon short-term semen preservation in chilled media, and, later, upon slightly longer-term preservation in ambient-temperature media [6,7,8,9]. In cattle, such methods were largely superseded by cryopreservation as LN2 became more widely available, but, in species that tolerate cryopreservation relatively poorly (e.g. the pig and the horse), chilled/ambient temperature preservation is still the dominant method of AI. Furthermore, in countries such as New Zealand that have a very high peak demand for bovine semen during the short breeding season, the ambient temperature diluent, Caprogen [10,11], has proved a satisfactory basis for a successful bovine AI service.

[27] established that Mpwapwa bulls’ sperm could survive for 48 - 72 h in the ambient temperature (AT) diluents Tris-egg yolk and Optixcell at temperatures of up to 32oC. In principle, such a period of survival in the ambient temperatures that pertain in the beef cattle-raising regions of Tanzania should be adequate for distribution to and insemination of the cows of smallholder farmers. However, survival of the semen *in vitro* is not, of course, evidence that it will achieve adequate pregnancy rates *in vivo*. To establish its efficacy *in vivo* an insemination trial is needed. A study was therefore undertaken to establish the feasibility of using low-cost ambient temperature semen system under Tanzanian condition and to determine the conception rates that could be achieved using AT semen in an oestrus-synchronisation fixed-time AI (FTAI) programme in Mpwapwa cows. This study is the first of its kind in Tanzania to test AT semen under field conditions. Alongside the feasibility study, the same number of cattle were inseminated using frozen semen after the same oestrus synchronisation programme, i.e. the standard AI approach which has been used by TALIRI Mpwapwa in the Mpwapwa district since the redevelopment of the TALIRI Mpwapwa laboratory (e.g. [5,12]). This was not designed as a direct comparison but a check to assess whether the fertility of the cattle given AT semen was likely to be similar to that of the cows used by TALIRI Mpwapwa in previous studies of AI.

2. material and methods

The study was conducted over two breeding seasons (April to September 2021 and April to September 2022). All animal-related manipulations had received Livestock Research Ethical Clearance from the Tanzanian Livestock Research Institute (TALIRI) (12/02/2021) prior to the start of the study.

Cow selection and allocation

All cows came from the TALIRI Mpwapwa research herd. Mature (≥2 years old) cows that were not pregnant and had no recorded history of previous reproductive problems, and which were not being used for other research programs, were eligible for selection. The number of cows selected in each year was based on the number of cows that was thought to be feasible to inseminate in four synchronised breeding groups. In 2021, 100 cows were selected for inclusion in the study and in 2022, 202 cows were selected.

Selected cows were ordered based on age, parity within age and ear tag number within parity within age, before being divided into four breeding groups (see Supplementary table 1), with the first cow going to Group 1, the second to Group 2, the third to Group 3 and the fourth to Group 4. This was then repeated until all cows were allocated. This meant that in 2021 there were 25 cows per breeding group and in 2022 there were 50 cows in Groups 1 and 2 and 51 in groups 2 and 4 (as the last two cows in the order were assigned to Groups 2 and 4). Groups 1 and 2 were assigned to receive the AT semen, and the remaining groups assigned to receive frozen semen.

Bull selection

One registered breeding bull was used across both seasons to produce the ambient temperature semen used for insemination and one bull was used to produce the frozen semen. To test the proof of concept of using AT semen, simply bulls were selected from the mature (≥2 years old) Mpwapwa breed bulls in the TALIRI Mpwapwa herd on the basis of having been shown to be suitable for use in AI [27] and having high individual sperm motility and ejaculate density (i.e. 90% and 873 sperm/mL for the AT semen bull, and 80% and 896 sperm/mL for bull producing frozen semen).

Animal health management and pre-breeding nutrition

All cows were negative from contagious abortion and all bulls were negative from *Tritrichomonas foetus* and *Campylobacter foetus.* Immediately after selection, animals were treated for internal parasites using either 0.5 ml/kg of a levamisole/oxyclozanide combination (nilfarm, farmers centre – Tanzania) (lactating cows only) or 50 µg/kg ivermectin (ivermectin, anglian nutrition product – UK) (dry cows and bulls). thereafter cows were separated by breeding group and given access to unrestricted grazing (stocking rate of 0.36 livestock unit/ha, principal grass species*: cenchrus ciliaris*, *Hyperrhenia rufa*, *Themeda spp, Cynodon dactylon* and *Chloris gayana*) for 4 weeks. Bulls were kept separately and given access to similar grazing. In addition to the pasture, cattle also received compound feed (0.3 kg/cow/day and 0.6 kg/bull/day) containing 600 g/kg maize bran, 390 g/kg sunflower seed cake, 10 g/kg salt), and had access to mineral lick blocks (Farmers Centres Ltd, Tanzania) (allocation rate of 200 g/cow/week and 400 g/bull/week). All animals were dipped weekly in a bath containing 100 g/L of alphacypermethrin (Paranex, Farm base Ltd, Tanzania) for the control of ectoparasites.

Allocation of cows to treatment

Oestrus synchronisation and AI protocol

The mating period for each breeding group for each year is summarised in Table 1. The same AI protocol was used in all groups in both years. On Day 0 (morning) each cow received 500 µg cloprostenol (Estroplan, Parnell, Australia) (PG1), followed by observation of behavioural oestrus for 96 h, aided by the use of one vasectomised teaser bull per group. Cows showing signs of oestrus were inseminated with AT or F semen, with timing based on the AM/PM rule [13]. Cows that were recorded as not having shown signs of oestrus were retreated with 500 µg of cloprostenol on Day 14 (PGF2α). Seventy-two hours after this 2nd injection, all retreated cows were inseminated (single insemination).

Semen processing, storage and handling for insemination

For AT semen, semen was collected from the selected bull in the afternoon on Days 1 and 15 after PG1. The collection on Day 15 was ~32 h after PGF2α. Semen was collected using electro-ejaculation (Ejakulator, Minitube, Tiefenbach, Germany) into a handled mounted semen collection cone (Minitube, Tiefenbach, Germany). The semen was then visually checked for volume and colour, with motility and morphology evaluated microscopically (MBL2000 Kruss Optronic GmbH, Germany) at x100 magnification (motility) and 1000x magnification (morphology) [14]. Concentration was determined using a previously calibrated spectrophotometer (Accuread Photometer, Biochrom Ltd, USA). Semen was then extended (1:1) using Optixcell diluent (IMV, L’Aigle, France), and packed and sealed into 0.25 mL Minitube ‘straws’ (Minitube, Tiefenbach, Germany) and held in a water bath at 20°C for up to two days (i.e. 48 h) before use.

The process of semen collection was the same for the frozen semen except that the diluent used also contained 7% v/v glycerol. The semen was then loaded and sealed into 0.25 mL straws at a temperature of 4°C before being frozen in liquid nitrogen vapour at a temperature of 125°C for 10 minutes, after which the straws were plunged into the liquid nitrogen. Prior to insemination, straws of frozen semen were thawed in water at 35oC for 2 minutes.

Management after AI

Cows were tested for pregnancy 2 months after FTAI using transrectal ultrasound (Chison Medical Imaging Co, Jiangsu, China), in order to determine whether they had conceived to AI. Non-pregnant cows were then grouped together and then separated into different three paddocks (i.e. 24/25 cows/ paddock of 3 ha) with each paddock allocated one bull on a ratio of 1:24 or 1:25. These bulls were kept with the cows for 60 days. At the end of that period of natural mating, transrectal pregnancy diagnosis was conducted. The number of non-pregnant cows was 18 (i.e. 14 in 2021 and 4 in 2022).

Statistical Method

For comparison between proportions of cows in each semen group responding to PG1 by showing oestrus, relative risk and confidence intervals were calculated according to the method of [15].

3. results and discussion

The overall conception rates achieved by AI over the two years of study were 62% (94/151) with AT semen and 38% (58/152) with frozen semen (Table 2). At the conclusion of the 60 days of bull mating after the FTAI, the final pregnancy rates were 96% (145/151) in the groups where AT semen had been used and 92% (140/152) where frozen semen had been used. This suggest that AT semen not only performs well in AI but also supports broader reproductive success.

The proportion of Mpwapwa cows which were observed in oestrus after PG1 was the same between the two groups (see Table 3), with, in both groups, ~⅓ of cattle responding to first PG; The relative risk for responding to PG1 in cows in the AT-semen groups versus those in the frozen-semen groups was 1.03 (95%CI: 0.75 1.41). Of the cows inseminated after PG1, 73% of cows given AT semen and 20% of cows inseminated with frozen semen became pregnant. The equivalent figures after PGF2α were 57% and 47% respectively.

Across both years the results achieved using cryopreserved semen were consistent with those reported by [5] who achieved a 39% conception rate in Mpwapwa cattle kept at the TALIRI Mpwapwa centre, following insemination with frozen semen after a double PG/FTAI protocol. Interestingly, in both the current study and in [5], the conception rates were appreciably lower than the 55% reported by [12] after a similar insemination regimen in communally-managed, mixed breed, zebu/zebu cross cattle owned by smallholders in villages close to TALIRI. These results indicate that the fertility of the cows in the current study was adequate and that the results from the AT semen were not artificially lowered because of underlying problems in the inseminated cows. Similarly, the response to PG1 (in terms of observedoestrus) in both AT and frozen semen groups inseminated (~33%) was better than the 10% response reported by [5], indicating that an unexpectedly poor responsiveness to PG was not a factor in determining the outcome of AT insemination. However, this warrants further investigation, employing multivariate analysis or regression modelling to identify factors such as body condition score, age, and reproductive history that influence AI success.

Over the two years of the study, the overall conception rate to the AT semen was 62%: results, which would be highly acceptable if repeated in routine AI service. Perusal of the literature revealed no similar studies reporting conception rates after AI with AT semen at PG-induced oestrus in zebu-type cattle in East Africa. However, similar outcomes (55% to 60%) have been reported in European cattle being managed under African conditions after AI with AT semen at spontaneous oestrus [16], while similar results (61%) have been reported in South American *Bos indicus* after AI with chilled semen following synchronisation with progesterone and oestradiol [17]. In the more temperate climate of New Zealand, AI with semen extended in the AT diluent Caprogen, gave a CR of 58% after single PG synchronization [18]. These results suggest that the results achieved in the present study using AT semen in combination with a double PG/FTAI may be routinely achievable. Nevertheless, larger scale studies are needed to better characterise the range of conception rates that are likely to be encountered in the Mpwapwa region when using AT semen.

As in previous Mpwapwa-based studies we synchronised cattle with a double PG/FTAI programme because, based on simplicity, cost and ease of scheduling, it is likely to be the synchronisation method used alongside AT semen in any AI programme. Across the two years of the study, oestrus behaviour was observed in 101 of the 303 cows (33%) after PG1. This was consistent across all four breeding groups, but lower than the 55% seen in smallholder-owned cattle in the Mpwapwa region [12], and higher than the 10% reported seen in Mpwapwa cattle at TALIRI Mpwapwa [5]. The disparity of these results strongly suggests that better information is needed on the factors driving the response to PG1 in cattle in the Mpwapwa region as these may be related to the success of the program. Indeed, for cows inseminated with AT-semen cows, the conception rate to AI at observed oestrus after PG1 was higher than to FTAI after PGF2α (73% versus 57%, respectively; relative risk 1.3; 95%CI 1.0 to 1.6), Thus, if we can reliably improve the proportion of cows showing oestrus after PG1, our data suggest that this should improve overall pregnancy rates.

If this combination of AT semen with a double PG/FTAI synchronisation is to be taken to beef farmers across central Tanzania, we do not just need acceptable rates, we need the process of creating AT semen to be relatively cheap simple and feasible. Thus, comparing between the steps involved in the production, storage and usage of AT and frozen semen is critically important. For both semen types, donorbulls have to be of satisfactory breeding soundness and to have semen that meets minimum criteria for use in AI, so there is no advantage for either type. Similarly, development of the semen straws is very similar with both AT and frozen semen being extended/diluted before being packed into straws. In the present study both F and AT semen were diluted 1:1 (i.e. the only difference being in the diluent which was Optixcell for AT semen and Optixcell +7%v/v glycerol for frozen semen). Thereafter, the AT semen was stored at ambient temperature for 2-3 days, whereas frozen semen was frozen using liquid nitrogen and maintained at 196oC. Prior to insemination, frozen semen needed to be thawed (i.e requiring additional kit), whereas AT semen could inseminated without further handling. These differences resulted in estimated costs per dose of approximately 20 USD (i.e. 50,000 TZS) for AT semen and approximately 40 USD (i.e. 100,000 TZS) for frozen semen. These costs were calculated based on the use of the low dilution rate of 1:1 for both AT and frozen semen, whereas, in reality, dilution rates could be considerably greater than this for field use (noting that AT semen can be diluted much further than frozen semen: [19]). Thus, overall, AT-semen is cheaper and easier to produce and use than frozen semen, even before taking into consideration the difficulties of procuring a consistent supply of liquid nitrogen. However, a comparative cost-benefit analysis of AT semen and F semen production, expressed as cost per dose, is recommended for future study. In Tanzania, inconsistent liquid nitrogen supply has been a major brake on the use of AI, especially in non-dairy regions [20,21,22]. Thus, developing an AT semen program at TALIRI Mpwapwa could result in the development of an AI program which is suitable for other centres across Tanzania which do not have access to liquid nitrogen.

The principal issue with AT semen is that it is not long lasting. Other data from TALIRI Mpwapwa show that survival for 48 hours is achievable for most bulls (although the semen of some bulls may last ≥72 hours) or more) [27]. However, further study is recommended to evaluate the field variability of AT semen under real distribution conditions, considering factors such as extreme temperatures and transportation time. This is the principal reason why we believe that any AI service using AT semen in Mpwapwa (or other similar regions) will be based on mass insemination after synchronisation, especially as in a survey of local smallholder cattle owners, of those owners who expressed a preference, 69% stated that if they were going to use AI they would prefer using a mass AI approach. The preference for this approach, further highlights the importance of optimising the re-sponse to such a programme, particularly optimising body condition score (BCS) and animal health. In the present study, cows underwent a 4-week preparation period in which they were treated for ecto- and endo-parasites and were fed good quality pasture and supplementary feed. This clearly improved BCS and thus is likely to have improved pregnancy rates. For example, [23] reported in a mixed FTAI/natural mating system better calving rates in *B. indicus* beef cattle with a BCS ≥5.0 than with BCS ˂5.0 (83.6% vs 73.3%, respectively). It is therefore axiomatic that, if an FTAI-based programme is introduced into the Mpwapwa district, it is not used as a substitute for inappropriate nutrition, poor quality herd health programs, or as a support for poor management [24,25,26]. In fact, introducing nutrition, fertility and herd health plans is likely to be a critical part of any successful AI program in the district.

Table 1. Mating plan for selected cows for the two breeding seasons

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Mating Groups | Cows/Group in 2021 (2022) | Semen Type | 1st PGF2α | Oestrus detection/AI | 2nd PGF2α | FTAI | Trans rectal US PD | Trans rectal US PD |
| 1 | 25 (50) | AT | 01 April | 3-6 April | 14 April | 17 April | 2021: 6 - 10 July  2022: 6 -15 July | 2021: 29-31 Oct  2022: 29 Oct - 02 Nov |
| 2 | 25 (51) | AT | 07 April | 9-12 April | 20 April | 23 April |
| 3 | 25 (50) | F | 13 April | 15-18 April | 26 April | 29 April |
| 4 | 25 (51) | F | 19 April | 21-24 April | 02 May | 05 May |

*AT: ambient temperature semen, F: frozen semen, AI: artificial insemination, FTAI: fixed-time artificial insemination, US: ultrasound PD: pregnancy diagnosis. Note: dates of PGF2α same in both years*

Table 2. Pregnancy rates to AI in Mpwapwa cows after oestrus synchronisation and FTAI with AT or F semen

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Breeding Season | Semen | | | |
| AT | | F | |
| Cows pregnant to AI (%) | Cows pregnant at end of breeding season (%) | Cows pregnant to AI (%) | Cows pregnant at end of breeding season (%) |
| Year 1 (2021) | 30/50 (60) | 45/50 (90) | 22/50 (44) | 41/50 (82) |
| Year 2 (2022) | 64/101 (63) | 100/101 (99) | 36/101 (35) | 99/101 (97) |
| Total | 94/151 (62) | 145/151 (96) | 58/151 (38) | 140/151 (92) |

*AT: ambient temperature semen, F: frozen semen*

Table 3. Comparison between AT-semen-inseminated and F-semen-inseminated cattle in: i) proportion given after 1st and 2nd PG injection and ii) proportion getting pregnant to inseminations after first and second PG injection

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Year 1\*** | | **Year 2\*** | | **TOTAL** | |
|  |  | AI | Pregnant | AI | Pregnant | AI | Pregnant |
| 1st PG injection† | AT | 21 (42%) | 15 (30%) | 30 (30%) | 22 (22%) | 51 (34%) | 37 (25%) |
| F | 18 (36%) | 5 (10%) | 32 (32%) | 5 (5%) | 50 (33%) | 10 (6%) |
| 2nd PG injection | AT | 29 (58%) | 15 (30%) | 71 (70%) | 42 (42%) | 100 (66%) | 57 (38%) |
| F | 32 (64%) | 17 (34%) | 70 (68%) | 31 (31%) | 101 (67%) | 48 (32%) |

*AT: ambient temperature semen, F: frozen semen. \*, n = 50 per semen type in year 1 and 101 per semen type in year 2. †, cows inseminated after 1st PG injection if seen in oestrus*

4. Conclusion

The conception rates seen in the current study after AI with AT semen in cows synchronised using a double PG protocol were consistently high across both years and consistent with results achieved using cryopreserved semen. If these results can be consistently obtained on smallholder farms in Tanzania, this protocol could be a significant addition to Tanzanian beef cattle breeding programmes. It should be noted that programme success also depends on sustainable AT semen supply and farmer training. Being relatively cheap and simple to schedule makes the programme accessible and implementable by the relatively poor semi-commercial smallholder farmers of the Mpwapwa district. Additional work is needed to optimise the conditions for making AT semen (i.e. diluent, dilution rate, whether or not to chill) and cows’ response to PG-based oestrus synchronisation (e.g. timing of PG treatments, body condition score, health status, plane of nutrition). Nevertheless, the present study clearly establishes the potential feasibility for an AI service based upon AT semen as a low cost means of breeding Mpwapwa and other beef cattle in the beef-raising areas of Tanzania. However, sustained policy support and cross-sector collaboration are crucial to ensure the long-term sustainability of this programme.

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Ethical approval

Research Ethical Clearance was obtained from the Tanzania Livestock Research Institute (TALIRI) (Feb 2022).

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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