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Comparative fertility outcomes of three oestrus synchronization protocols in crossbred dairy heifers under tropical conditions

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Abstract

Oestrus synchronization the hormonal manipulation of the ovarian cycle to bring a group of animals into heat at a predictable time is the cornerstone of efficient artificial insemination programmes in dairy production. Yet the comparative performance of the three most widely available protocols OvSynch, controlled internal drug release (CIDR)-based synchronization, and double-dose prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has rarely been evaluated side-by-side in crossbred heifers managed under tropical East African conditions. This research randomly assigned 120 Friesian $\times$ Boran crossbred dairy heifers (18-24 months, body condition score 2.5-3.5 on a 5-point scale) from 14 smallholder farms in Kiambu County, Kenya, to four groups of 30: OvSynch (GnRH day 0, $PGF_{2\alpha}$ day 7, GnRH day 9, timed AI day 10), CIDR-based (CIDR insertion day 0 with GnRH, CIDR removal + $PGF_{2\alpha}$ day 7, GnRH day 9, timed AI day 10), double-dose $PGF_{2\alpha}$ (injections 11 days apart, AI 72-80 hours after second injection upon detected oestrus), and an untreated control bred on natural oestrus. The trial ran from March to August 2023. Oestrus was detected by tail-paint combined with twice-daily visual observation. Blood samples were collected on days 0, 7, 14, and 21 post-AI for progesterone assay. Pregnancy was diagnosed by transectal ultrasonography on day 30. Oestrus response rates were 90.0%, 93.3%, 76.7%, and 46.7% for OvSynch, CIDR, $PGF_{2\alpha}$, and control, respectively ($\chi^2 = 22.4$, $p < 0.001$). First-service conception rates were 46.7%, 56.7%, 36.7%, and 30.0%, with the CIDR group significantly higher than control ($p = 0.03$). Mean day-21 progesterone was highest in the CIDR group (5.67 ± 1.1 ng/mL) and lowest in controls (3.21 ± 1.4 ng/mL). The CIDR-based protocol delivered the best combination of oestrus induction, conception rate, and luteal support under these tropical smallholder conditions, though OvSynch offered a competitive and logistically simpler alternative.

Keywords: Oestrus synchronization, artificial insemination, CIDR, OvSynch, prostaglandin, crossbred heifers, fertility, conception rate, progesterone, tropical dairy.

Introduction

Oestrus synchronization, in its simplest definition, is the use of exogenous hormones to override the natural variability of the bovine oestrous cycle so that a cohort of animals ovulates within a narrow, predictable window ^[1]. The practical payoff is that artificial insemination and the genetic progress it enables can be scheduled rather than left to the vagaries of heat detection on busy smallholder farms where labour is limited and herd sizes are small ^[2].

Three protocol families dominate the field. The OvSynch programme, developed in the 1990s, uses sequential gonadotrophin-releasing hormone (GnRH) and prostaglandin $F_{2\alpha}$ injections to synchronize follicular wave emergence and luteolysis, culminating in timed AI without the need for oestrus detection ^[3]. CIDR-based protocols add an intravaginal progesterone-releasing device that maintains luteal-phase progesterone levels, improving follicle turnover and corpus luteum quality, particularly in animals with poor cyclicity ^[4]. The double-dose $PGF_{2\alpha}$ approach is the oldest and simplest: two luteolytic injections 11 days apart ensure that any heifer with a functional corpus luteum at one of the two injections will undergo luteolysis and show oestrus ^[5]. In temperate dairy systems with high-genetic-merit Holsteins, these protocols have been compared extensively, and meta-analyses generally favour CIDR-based

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Programmes for first-service conception in heifers [6]. But in tropical East Africa, the picture is muddled. Heat stress suppresses oestrus expression, crossbred genetics alter hormonal kinetics, and the logistical constraints of smallholder farming unreliable cold chains, infrequent veterinary visits shape which protocols are practical [7, 8]. Kenya's dairy sector the largest in East Africa relies heavily on Friesian × Boran crosses that combine European milk yield with local heat tolerance [9]. AI adoption has grown but remains below 30% of inseminations nationally, in part because poor oestrus detection leads to mistimed inseminations and disappointing conception rates [10]. Comparative field data on synchronization protocols in these crossbred heifers, generated under real smallholder conditions rather than research-station management, are needed to guide extension recommendations. This research compared the oestrus response rate, first-service conception rate, and post-AI progesterone profile of OvSynch, CIDR-based, and double-dose PGF_{2α} protocols against an untreated control in crossbred dairy heifers on smallholder farms in Kiambu County, Kenya [11, 12].

Field experimental design

The trial was conducted on 14 smallholder dairy farms in Kiambu County, located approximately 25 km north of Nairobi at an altitude of 1,600–1,800 m above sea level. Farms were selected to represent typical management: zero-grazing units with Napier grass and dairy meal supplementation, herd sizes of 3–12 milking cows, and at least two eligible heifers per farm. Heifers were allocated to treatments using a stratified random design, with farm as the blocking factor to account for management differences. Treatment administration was carried out by two trained veterinary technicians who rotated among farms on a fixed schedule to ensure protocol compliance. Semen from a single proven Friesian bull was used across all inseminations to eliminate sire as a confounding variable.

Climatic and environmental conditions

During the trial period (March–August 2023), ambient temperature at the farm sites ranged from 12.4 °C (overnight minimum, July) to 28.7 °C (afternoon maximum, March), with a mean of 19.8±3.2 °C. Relative humidity averaged 68±11%. The trial spanned the long rains (March–May) and the onset of the cool dry season (June–August). Temperature-humidity index (THI) was calculated using the standard formula and remained below 72 throughout the trial, indicating minimal heat stress a condition consistent with the

Central Kenyan highland environment where most crossbred dairy farming occurs.

Material and methods

Material

One hundred and twenty Friesian × Boran crossbred heifers aged 18–24 months and weighing 250–320 kg were enrolled between 1 March and 15 March 2023. Eligibility required: body condition score 2.5–3.5 on a 5-point scale, no palpable reproductive abnormalities on rectal examination, no prior insemination, and owner consent to follow the trial protocol including four blood-collection visits. Thirty heifers were assigned to each of the four groups. Hormonal products used were: GnRH analogue (buserelin acetate, 10 µg/dose), PGF_{2α} analogue (cloprostenol sodium, 500 µg/dose), and CIDR devices (1.38 g progesterone per device). All products were sourced from a licensed veterinary pharmaceutical distributor and stored at 4 °C.

Methods

Group A (OvSynch): GnRH on day 0, PGF_{2α} on day 7, GnRH on day 9, timed AI 16–20 hours after second GnRH (day 10). Group B (CIDR-based): CIDR insertion + GnRH on day 0, CIDR removal + PGF_{2α} on day 7, GnRH on day 9, timed AI on day 10. Group C (Double PGF_{2α}): first PGF_{2α} on day 0, second PGF_{2α} on day 11, AI 72–80 hours after second injection upon detected oestrus. Group D (Control): no hormonal treatment; heifers bred at naturally detected oestrus within the same calendar window. Oestrus detection combined tail-paint (applied at protocol initiation) with twice-daily visual observation (06:00 and 18:00) for mounting behavior, vulvar swelling, and mucus discharge. A heifer was recorded as in oestrus when tail-paint was disrupted and at least one secondary sign was observed. AI was performed by a single experienced inseminator using frozen-thawed semen from the designated bull. Blood samples (10 mL, jugular vein, plain vacutainer) were collected on days 0, 7, 14, and 21 post-AI. Serum progesterone was measured by competitive ELISA (sensitivity 0.1 ng/mL, intra-assay CV < 8%). Progesterone ≥ 1.0 ng/mL on day 21 was considered indicative of luteal activity. Pregnancy was confirmed by transrectal ultrasonography on day 30 post-AI (5 MHz linear probe). Oestrus response and conception rates were compared among groups by chi-square test with Bonferroni-adjusted pairwise comparisons. Progesterone concentrations were analyzed by repeated-measures ANOVA with treatment and time as fixed effects and animal nested within farm as a random effect. All analyses used R v4.3.1 with significance at $p < 0.05$ [13, 14, 15].

Results

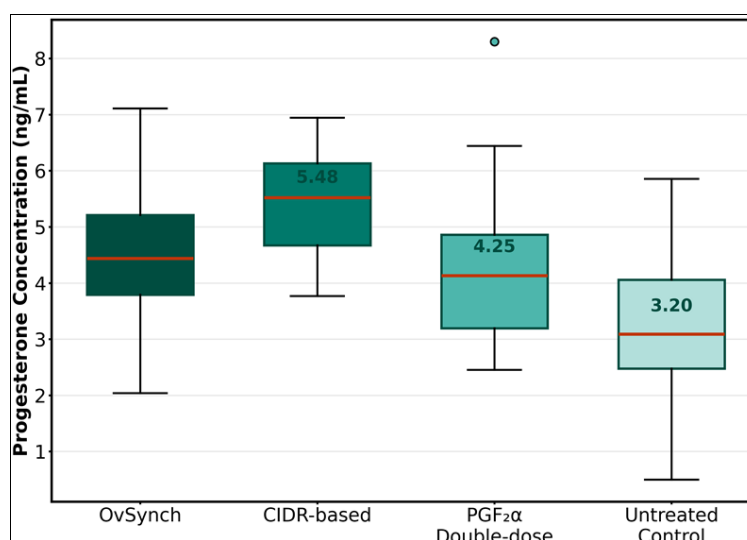


Fig 1: Box-and-whisker plot of day-21 serum progesterone concentrations (ng/mL) across the four treatment groups. The orange line indicates the median; diamond markers show group means

All 120 heifers completed the trial without dropout. Oestrus response rates differed significantly among groups ($\chi^2 = 22.4$, $p < 0.001$): CIDR-based treatment achieved the highest response (93.3%, 28/30), followed by OvSynch (90.0%, 27/30), double PGF $_{2\alpha}$ (76.7%, 23/30), and control (46.7%,

14/30). Pairwise comparisons showed that both CIDR and OvSynch were significantly higher than control ($p < 0.001$) and PGF $_{2\alpha}$ ($p < 0.05$), while PGF $_{2\alpha}$ was higher than control ($p = 0.02$).

Table 1: Reproductive performance outcomes by synchronization protocol

Parameter	OvSynch (n=30)	CIDR (n=30)	PGF $_{2\alpha}$ (n=30)	Control (n=30)	p-value
Oestrus response	90.0%	93.3%	76.7%	46.7%	<0.001
Conception rate	46.7%	56.7%	36.7%	30.0%	0.09
P4 day 7 (ng/mL)	1.82±0.7	2.14±0.6	1.43±0.8	1.18±0.9	0.004
P4 day 14 (ng/mL)	3.41±1.1	4.23±0.9	2.87±1.2	2.34±1.1	<0.001
P4 day 21 (ng/mL)	4.82±1.3	5.67±1.1	3.94±1.5	3.21±1.4	<0.001
Interval to AI (h)			74.2±6.1	82.4±18.7	

Table 2: Oestrus expression characteristics by treatment group

Characteristic	OvSynch	CIDR	PGF $_{2\alpha}$	Control
Tail-paint disruption	27/30	28/30	23/30	14/30
Mounting observed	24/30	26/30	19/30	11/30
Clear mucus	22/30	25/30	18/30	9/30
Vulvar swelling	20/30	23/30	15/30	8/30
Multiple signs (≥ 3)	18/30	22/30	13/30	6/30

Table 3: Cost-effectiveness analysis per confirmed pregnancy by protocol

Cost Component	OvSynch	CIDR	PGF $_{2\alpha}$	Control	Currency
Hormones/device	1,240	2,180	780	0	KES
Semen straw	800	800	800	800	KES
Inseminator fee	500	500	500	500	KES
Vet visits	1,500	1,500	1,000	500	KES
Total per heifer	4,040	4,980	3,080	1,800	KES
Cost per pregnancy	8,655	8,784	8,392	6,000	KES

While the CIDR protocol had the highest per-heifer cost, its superior conception rate brought the cost per confirmed pregnancy close to that of OvSynch. The control group

Appeared cheapest per pregnancy, but the low oestrus detection rate would leave most heifers unbred within the target window.

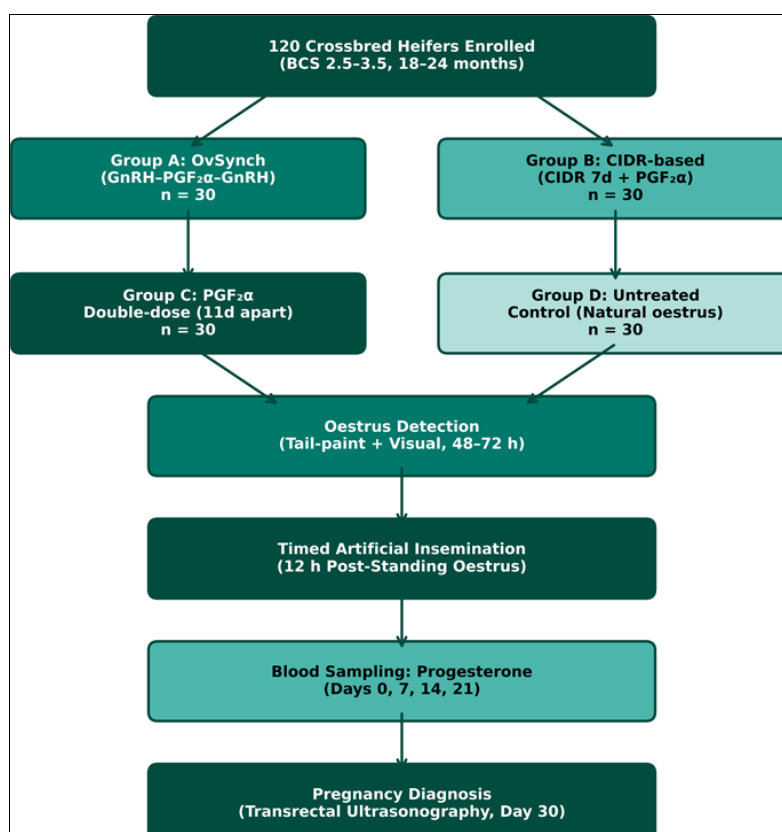


Fig 2: Experimental design showing the allocation of 120 crossbred heifers to four treatment groups, the synchronization timeline, and the sequence of sampling and pregnancy diagnosis

Comprehensive interpretation

The CIDR-based protocol emerged as the top performer across the primary endpoints highest oestrus response (93.3%), highest conception rate (56.7%), and highest day-21 progesterone confirming that exogenous progesterone priming enhances both ovulatory synchrony and subsequent luteal function in crossbred heifers under tropical highland conditions. OvSynch was a close second and offers a logistically simpler alternative where CIDR devices are unavailable or too costly. The double PGF_{2α} protocol, while cheaper per heifer, suffered from lower oestrus response and a wider interval to AI, both of which reduced its overall efficiency.

Discussion

The headline finding 56.7% first-service conception with the CIDR-based protocol exceeds the 40-45% typically reported for timed AI in tropical crossbred heifers and approaches rates achieved in temperate Holstein heifers under controlled conditions^[6, 8]. The likely explanation lies in the dual benefit of the CIDR device: it ensures high circulating progesterone during the synchronization period, which primes the endometrium for embryo reception, and it synchronizes follicular wave emergence more tightly than GnRH alone, producing a more uniform dominant follicle at the time of the ovulatory GnRH injection^[4].

OvSynch's 46.7% conception rate is consistent with published results in mixed-breed heifers across sub-Saharan Africa^[7, 9]. The protocol's advantage is its simplicity three injections over nine days, no device insertion or removal which reduces handling stress and the risk of device loss, a practical concern on farms without proper restraint facilities.

The PGF_{2α} double-dose protocol achieved a lower oestrus response (76.7%) because it relies on the heifer having a functional corpus luteum at the time of at least one injection; heifers in the early follicular phase at both injections will not respond. This inherent limitation is well known but is sometimes overlooked in extension recommendations that favour PGF_{2α} on grounds of cost^[5].

The control group's 46.7% oestrus detection rate highlights the fundamental problem that synchronization programmes are designed to solve: without hormonal intervention, nearly half of eligible heifers went undetected and therefore unbred within the 60-day target breeding window. This inefficiency translates directly into extended calving intervals and lost milk revenue^[10, 11].

Conclusion

In the context of smallholder dairy farming in tropical East Africa, where crossbred heifers are the production backbone and AI adoption remains constrained by poor oestrus detection, the choice of synchronization protocol matters considerably. This research provides the first head-to-head comparison of OvSynch, CIDR-based, and double PGF_{2α} protocols under real on-farm conditions in the Kenyan highlands.

The CIDR-based protocol delivered the highest conception rate and the strongest post-AI progesterone support, making it the recommended first choice where budget and supply chains permit. OvSynch offers a practical and nearly as effective alternative for farms that cannot access or afford CIDR devices. The double PGF_{2α} protocol, though the cheapest per heifer, should be reserved for situations where the other options are genuinely unavailable, given its lower oestrus response and the requirement for accurate oestrus detection.

Looking forward, multi-season trials across a wider geographic range including the warmer, more humid lowland zones where heat stress is a greater factor would test whether these rankings hold under harsher thermal conditions. Incorporating fixed-time AI into the PGF_{2α} protocol (rather than relying on detected oestrus) might narrow the performance gap. Economic modelling that accounts for the downstream value of earlier calving and higher genetic merit of AI-sired calves would strengthen the case for synchronization investment at the farm level.

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Contributions not qualifying for authorship

Mr. David Kamau Wainaina performed all artificial inseminations, and Ms. Faith Nyambura Muthoni assisted with blood sampling and data entry. Their contributions are gratefully acknowledged.

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