Research Report

Early pregnancy outcomes in East African Shorthorn Zebu cattle synchronized with 5 progesterone-based Co-synch protocol modifications

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Abstract

Estrus and ovulation synchronization optimize artificial insemination and accelerate livestock productivity improvement by enhancing reproductive performance. However, available protocols are suboptimal in *Bos indicus* cattle, justifying search for novel protocols. We evaluated the effects of 5 synchronization protocols on conception rates in 232 Shorthorn Zebu cows. The protocols were: 7-day Co-synch + progesterone intravaginal device (P_4ID); n = 40); Bee-Synch I (n = 44); Bee-Synch II (n = 42); Mak-Synch I (modification of 7-day Co-synch + P_4ID that extends the time for GnRH-2 and timed AI from 60-66 to 74-78 hours, respectively; n = 52); and Mak-Synch II (a modification of Bee Synch II that extends the time for GnRH-2 and timed AI from 64-68 to 74-78 hours, respectively; n = 54). Pregnancy status was determined on day 32 via transrectal ultrasonography. A multiple logistic regression model was used to compare mean conception rates among protocols at the 95% confidence level in R. Adjusted mean (± standard error) conception rates were 44.59 ± 9.38%, 37.26 ± 8.48%, 42.09 ± 8.86%, 57.84 ± 8.42% and 47.19 ± 7.76% for 7-day Co-synch + P_4ID, Bee-Synch I, Bee-Synch II, Mak-Synch I and Mak-Synch II protocols, respectively, not different ($p > 0.05$) among protocols. Compared to cows with body condition score (BCS) 2.5, there were significantly higher odds of conception in those with BCS 3.0 (OR = 4.24, 95% CI: 1.19-20.2, p = 0.039), BCS 3.5 (OR = 5.45, 95% CI: 1.46-26.8, p = 0.019) and BCS 4.0 (OR = 5.79, 95% CI: 1.34- 31.7, p = 0.026). Results highlighted the importance of good nutrition that ensures cows achieve a BCS of at least 3 (scale: 1-5) before breeding.

Keywords: Zebu cattle, estrus, synchronization, conception

Introduction

Livestock production is an important sector in Uganda because it is a source of livelihood for majority (58%) of country's households.[1] Cattle are the most important livestock in Uganda, as they contribute up to 73% of the gross livestock value through beef and milk. Despite its importance, the country's livestock productivity remains low and accounts for only 3.5% of the gross domestic product.[1] Poor animal genetics could be responsible for the low productivity, as 93.5% of Uganda's cattle population is comprised of indigenous *Bos indicus* cattle types, mainly the East African Shorthorn Zebu (SHZ) and the Ankole longhorn cattle breeds.[1] These *B. indicus* breeds are known to be naturally characterized by low milk yields, slow growth rates and low carcass weights.[2,3]

Animal genetic improvement through selective breeding among *B. indicus* and crossbreeding *B. indicus* with high-grade *B. taurus* cattle could offer a sustainable solution to cattle productivity challenges in Uganda and Africa at large. Crossbreeding *B. indicus* with *B. taurus* has been proven to improve animal productivity in tropical countries by

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combining climate and disease adaptation of *B. indicus* breeds with high productivity traits of *B. taurus* breeds in the offspring, while taking advantage of heterosis.[4] Due to its efficiency and affordability in the African context, artificial insemination (AI) is the best technology for improving cattle genetics.[5,6] Incorporation of estrus synchronization in AI programs can further enhance reproductive performance and herd genetic improvement rate,[7] by eliminating challenges associated with AI (e.g. estrus detection, timing of ovulation) and by enabling breeding of a large number of cows at once.[8,9]

Most estrus and ovulation synchronization protocols have produced acceptable pregnancy rates in *B. taurus* but not *B. indicus* cows and heifers.[10-13] The existence of significant physiological and endocrinological differences between *B. taurus* and *B. indicus* cattle types has been advanced as an explanation for observed differences in protocol effectiveness. These widely reported differences include estrus duration, estrus to ovulation interval, incidences of silent and split estrus, and anestrus.[13,14] This motivated a further search for effective estrus and ovulation synchronization in *B. indicus* breeds. Recently, based on studies on ovarian follicular dynamics and conception rates in *B. indicus* x *B. taurus* crossbreds in the United States, 2 new protocols, Bee synch I and II were developed and reported to be more effective in *B. indicus* cows.[15,16] These protocols were however tested only in *B. taurus* x *B. indicus* crossbreed cattle in subtropical climates of the southern United States. Information is lacking on the performance of these protocols on purebred *B. indicus* cattle genotypes (e.g. SHZ cattle). The difference in climates and management realities in tropical Africa could also impact the effectiveness of such synchronization protocols.

In a recent study on ovarian follicular dynamics in SHZ cows synchronized with 7-day Co-synch + P_4ID, Bee synch I and Bee synch II protocols,[17] differences among protocols in preovulatory follicle diameters as well as ovulation rates and intervals between estrus onset and ovulation were reported. These differences could have serious implications for the development of effective protocols for synchronizing estrus and ovulation in SHZ cattle. However, that study did not evaluate downstream reproductive outcomes (e.g. conception rate), which is very important and indispensable for the practical evaluation of the effectiveness of any estrus and ovulation synchronization protocol. The same study reported mean ovulation timings from P_4ID withdrawal, 98 hours for the 7-day Co-synch + P_4ID and 97 hours for the Bee synch II protocol, and it recommended delaying timed AI (TAI) for both protocols. This suggestion was based on the recommendation that the best time for AI is 7-18 hours before ovulation.[18] Therefore, the current study aimed to evaluate the effects of the 7-day Co-synch + P_4ID, Bee synch I, Bee synch II, a modified 7-day Co-synch + P_4ID (Mak-synch I) and a modified Bee synch II (Mak-synch II) protocols on conception of SHZ cows on extensive management. We hypothesized that delaying TAI and the last GnRH treatment improve conception rates by providing an adequate period for additional growth of the dominant follicle before its stimulation to ovulate by the last exogenous GnRH. This study therefore tested the hypothesis that reducing the P_4ID implant duration from 7 to 5 days with (Bee synch I) or without (Bee synch II) 2 additional doses of $PGF_{2\alpha}$ 1 at protocol onset and another at P_4ID withdrawal; or delaying TAI from between 60-66 hours (7-day Co-synch + P_4ID) to between 74-78 hours (Mak-synch I) or from 64-68 hours (Bee synch II) to 74-78 hours (Mak-synch II protocol) improves conception rates in SHZ cows.

Materials and methods

Study location

The study was conducted from August 2023 to April 2024 on Lusenke Stock Farm, a government-owned livestock farm managed by the National Animal Genetic Resources Centre and Data Bank. The farm is located in Kayunga District in Central Uganda (Latitude 0.882827, longitude 32.983797, altitude 1048 meters) and had an ambient temperature of 16-30°C and an average precipitation of 100.7-232.8 mm during study period.

Experimental subjects

Animals were postpartum suckled SHZ cows that were maintained on an extensive ranch management system. Paddocks where the cows grazed mostly contained star grass (*Cynodon nlemfuensis*), *Chloris gayana*, and *Pennisetum purpureum*, with water points and tree shades in place. Cows in the paddocks had access to the Vitaphos Maziwa mineral block (Cibus Animal Nutrition Ltd, Nairobi, Kenya) throughout the research. On 3-month routines, the farm used anthelmintic deworming and trypanosomiasis prophylaxis. Weekly acaricide applications were used in a spray race for tick management. Cows in the experiment were selected from herds that were screened and free of brucella, campylobacter and trichomonas pathogens. Cattle breeding on the farm was exclusively by AI, either through estrus observation or estrus and ovulation synchronization.

All cows considered for the study had a transrectal ultrasonographic examination 7 days prior to synchronization treatments and were confirmed nonpregnant and having a normal, fully involuted uterus. A metricheck probe was used to detect and eliminate cows with metritis. Cows' cyclicity status and estrous cycle phase were not examined prior to experiment. Data on body condition score (BCS) and body weights were recorded 1 day before the initiation of the experimental treatments. The BCS were measured on a scale of 1-5, where 1 meant very thin and 5 very fat.[19]

Experimental design

A total of 242 East African Shorthorn Zebu cows were initially enrolled and randomly allocated to 5 treatment groups corresponding to 7-day Co-synch + P_4ID (n = 44), Bee Synch I (n = 46), Bee Synch II (n = 44), modified 7-day Co-synch + P_4ID (Mak Synch I, n = 53), and modified Bee Synch II (Mak Synch II, n = 55) from September 2023 to March 2024. The sample size was arrived at using power analysis,[20] at a 95% level of confidence, 80% statistical power, and using an effect size of 0.25 and the sample size was corrected for attrition.[21] During the course of the experiment, 10 cows were excluded due to management-related reasons that rendered them unsuitable for TAI. Specifically, these affected cows either missed or were presented outside the planned synchronization and TAI windows. Consequently, a total of 232 cows completed the experiment. The distribution of experiment subjects by protocol and climatic season is presented (Table 1).

Estrus and ovulation synchronization protocols

Cows in the 7-day Co-synch + P_4ID group received 100 µg of gonadorelin (Cystorelin, Ceva, Libourne, France)

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Table 1. Allocation of cows into experimental groups

Month/Year	Climatic season	7-day Co-synch + P_4ID	Bee Synch I	Bee Synch II	Mak Synch I	Mak Synch II	Total
September 2023	Rainy	10	–	12	18	27	67
November 2023	Dry	18	18	–	–	15	51
December 2023	Dry	–	–	13	10	12	35
March 2024	Rainy	12	26	17	24	–	79
Total		**40**	**44**	**42**	**52**	**54**	**232**

concomitantly with P_4ID implant (PRID delta, Lenexa, containing 1.55 g of progesterone) on day 0. This was followed by 25 mg of dinoprost (Enzaprost, Ceva, Libourne, France) on day 7, concurrent with P_4ID withdrawal. An additional dose (100 µg) of gonadorelin was given at AI, 62-64 hours from P_4ID withdrawal.[10,15] For the Bee synch I group, cows received 100 mg of gonadorelin and 25 mg of dinoprost alongside P_4ID insertion on day 0. This was followed by a double dose (50 mg) of dinoprost on day 5, concurrent with P_4ID withdrawal. A second dose of gonadorelin (100 µg) was given at AI, 65-66 hours from P_4ID withdrawal.[16] For the Bee synch II cows, 25 mg of dinoprost was given with P_4ID insertion on day 0, and followed on day 5, with 1 dose (25 mg) of dinoprost given at P_4ID withdrawal. A dose (100 µg) of gonadorelin was given at AI, 65-66 hours after P_4ID withdrawal.[16] The Mak-synch I group received the 7-day Co-synch + P_4ID treatment, except that the second dose (100 µg) of gonadorelin (GnRH-2) was given 74-78 hours after P_4ID withdrawal, together with AI. The Mak-synch II group received the Bee synch II protocol, except that the second dose (100 µg) of gonadorelin (GnRH-2) was given 74-78 hours after P_4ID withdrawal, together with AI. Intramuscular injections of gonadorelin and dinoprost were given in the neck and P_4ID were inserted into the vagina using a progesterone intravaginal device applicator (PRID applicator, Ceva, Libourne, France). The days mentioned are in reference to the day of the start of each synchronization exercise, considered as day 0 (Figure 1). The treatment of subjects in each of the 5 experiment groups was conducted serially rather than together to provide adequate time for TAI.

Artificial insemination quality assurance

All semen straws used in the study were from 1 Tyrolean Grey bull. The viability of the semen was evaluated prior to insemination by conducting a post-thaw motility evaluation under a light microscope. The semen batches used had a percent progressive motility of at least 60%. All inseminations in this study were performed by 1 technician.

Pregnancy diagnosis

Cows were examined via transrectal ultrasonography on day 32 after insemination.[22] An ultrasound scanner (Anyscan Multi, SONGKANG GLC Co ltd, Seongnam City, South Korea) equipped with a 6.5 MHz rectal linear probe was used. All scans were performed by 1 operator who scanned the same area at least twice before making a diagnosis. A positive diagnosis of pregnancy was made on visualization of an embryo as an echoic mass close to the uterine wall, inside a zone of anechoic uterine fluid, coupled with the presence of a corpus luteum on the ovary ipsilateral to the uterine horn containing the embryo. Conception rate was calculated as the total number of pregnant cows per total number of cows inseminated, multiplied by 100.[23]

Data analyses

The raw data were entered into a Microsoft Excel spreadsheet and later exported to R version 4.4.2.[24] Descriptive statistics, including graphs, were generated using R Studio. For each outcome variable, data for each protocol group were visualized using histograms and density plots and measures of central tendency and dispersion were obtained using the describe function from the psych package in R.[25]

A multiple logistic regression model was used to determine the effect of protocol type on conception while controlling for other factors, using the GLM function from the stats package in R,[24] and the family = binomial and link = logit, to compare their mean values across protocols at 95% confidence level. During initial analyses, an ANOVA (likelihood ratio) test was performed between a complex model with interaction terms for season and a simpler model with no interaction terms to determine if the added complexity was statistically warranted. The interaction terms were subsequently removed since there were no significant interaction effects. The simplest model, GLM (conception ~ protocol + parity + BCS + body weight) that had the lowest Akaike Information Criterion (AIC) value, was retained after examining for model fitness using the Simulate Residuals function in the DHARMa package in R.[26] Post hoc pairwise comparisons were conducted using the pairs and Emmeans functions from the Emmeans package in R,[27] to compute estimated marginal means, and the adjust = non and adjust = holm were used to obtain unadjusted and adjusted p-values, respectively.

Results

Body weights, parities and body condition scores of cows

Overall, the body weights of the experimental cows ranged 190-386 kg, with a mean (± standard error) of 269.27 ± 2.48 kg. Mean weights of cows in each of the 5 treatment groups were normally distributed ($p > 0.05$) and were 263.07 ± 4.52, 267.53 ± 6.30, 276.12 ± 6.08, 268.77 ± 5.69 and 276.18 ± 5.48 kg for 7-day Co-synch + P_4ID, Bee Synch I, Bee Synch II, Mak Synch I and Mak Synch II groups, respectively. There were no significant differences in mean body weights among treatment groups ($F [4, 231] = 0.964$, $p = 0.428$). Cow parities were categorized into primiparous (parity 1) and multiparous (parities 2-5). Overall, 40.1% (93/232) cows were primiparous and 59.9% (139/232) were multiparous cows. Cow BCS were 2.5 (6.5%), 3.0 (46.1%), 3.5 (31.0%) and 4.0 (16.4%).

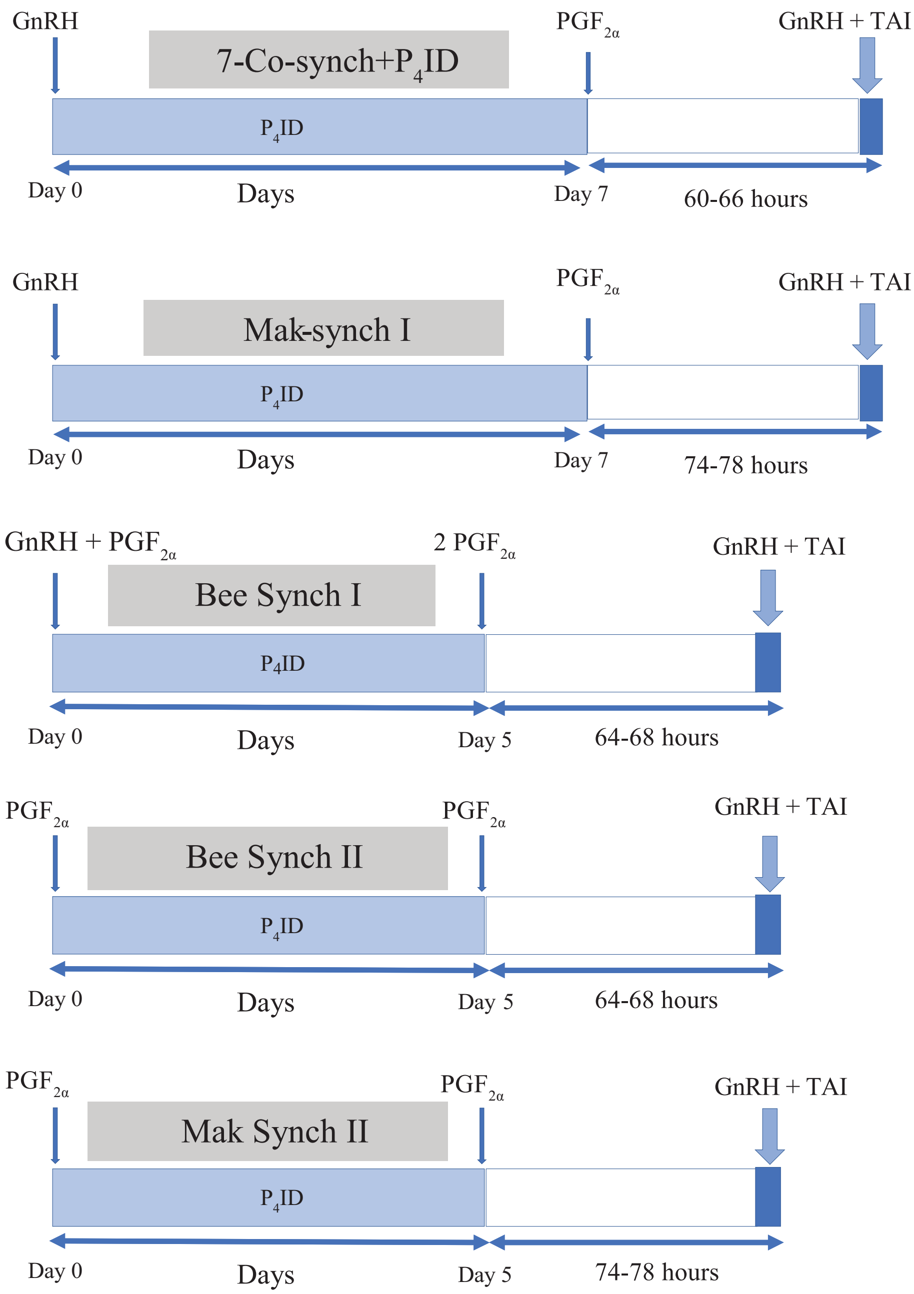

Figure 1. Estrus and ovulation synchronization treatment protocols

Conception rates

Conception rates, unadjusted and adjusted for parity, BCS and body weight, are presented (Table 2). Unadjusted conception rates ranged from 45.45% in the Bee-synch I group to 65.38% in the Mak-synch II group, whereas adjusted conception rates ranged from 37.26% in the Bee-synch I group to 57.84% in the Mak Synch I group. Although conception was generally low in adjusted verses unadjusted values, the trend in the differences in conception rate among protocols was maintained. In both cases, conception rate was highest in the Mak-synch I protocol and lowest in the Bee Synch I protocol, and Mak-synch I and Mak-synch II protocols resulted in higher conception rates than their respective original protocols; 7-day Co-synch + P_4ID and Bee-synch II.

Effect of estrus synchronization protocols on conception of cows

A multiple logistic regression model (Table 3) to predict conception based on protocol, BCS, parity and body weight (formula: GLM (conception ~ protocol + BCS + parity + body weight, family = binomial) revealed that synchronization protocols did not differ significantly in conception outcomes relative to the 7-day Co-Synch + P_4ID reference, although Mak-synch I had a tendency for higher odds (OR = 1.70, 95% CI: 0.70-4.21, p = 0.242). Although the global model indicated no significant overall effect of synchronization protocol on conception rates, protocol was the primary variable of interest in this study. Therefore, pairwise comparisons were conducted to further explore potential differences between

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Table 2. Mean conception rates of cows, unadjusted and adjusted for cow parity, BCS and body weight

Protocol	# Cows	Unadjusted CR (%)	Adjusted CR (%)	95% CI
7-day Co-synch + P_4ID	40	52.5	44.59	27.67-62.88
Bee synch I	44	45.5	37.26	22.57-54.74
Bee synch II	42	52.4	42.09	26.23-59.66
Mak-synch I	52	65.4	57.84	41.07-72.97
Mak-synch II	54	51.9	47.19	32.68-62.19

Table 3. Effects of synchronization protocols, BCS, parity and body weight on odds of conception

Predictor	aOR	SE	z-value	p-value	95% CI
Intercept	0.02	1.30	-3.15	0.00	0.00-0.2
Protocol (Reference = 7-day)					
Bee synch I	0.74	0.47	-0.65	0.52	0.29-1.85
Bee synch II	0.90	0.47	-0.22	0.82	0.36-2.26
Mak-synch I	1.70	0.46	1.17	0.24	0.7-4.21
Mak-synch II	1.11	0.46	0.23	0.82	0.45-2.74
BCS (Reference = 2.5)					
BCS 3.0	4.24	0.70	2.06	0.04	1.19-20.23
BCS 3.5	5.45	0.72	2.35	0.02	1.46-26.84
BCS 4.0	5.79	0.79	2.23	0.03	1.34-31.71
Parity (Reference = Primiparous)					
Pluriparous	1.69	0.29	1.78	0.07	0.95-3.01
Body weight (Reference = 0 kg)					
Body weight	1.01	0.00	2.06	0.04**	1.00-1.02

1 *p < 0.05; **p < 0.01; ***p < 0.001

aOR = adjusted Odds Ratio, CI = Confidence Interval of aOR, SE = standard error of aOR

Model = glm (Conception ~ Protocol + Parity + BCS + Body weight, family = binomial), AIC = 315.58

protocols. Pairwise comparisons between protocols did not reveal significant differences in conception rates. However, it is noteworthy that the comparison between Bee-Synch I and Mak-synch I approached significance (aOR = 0.43, 95% CI: 0.18-1.02, p = 0.056), suggesting a potential tendency toward higher conception with Mak-synch I, although this did not reach significance.

Effects of cow parity on conception

Pluriparous cows tended to have higher odds of conception compared to primiparous cows among 5 protocols (Table 3). However, this observed trend was marginally nonsignificant (OR = 1.69; 95% CI: 0.95-3.00; p = 0.07).

Effects of cow body condition score on conception

In the multivariable logistic regression model, body condition score (BCS) was a significant predictor of conception. Compared to cows with BCS 2.5, those with BCS 3 (OR = 4.24, 95% CI: 1.19-20.2, p = 0.039), BCS 3.5 (OR = 5.45, 95% CI: 1.46-26.8, p = 0.019), and BCS 4 (OR = 5.79, 95% CI: 1.34-31.7, p = 0.026) had significantly higher odds of conceiving. Therefore, under-conditioned cows (BCS 2.5) had reduced fertility compared to cows in moderate condition (BCS 3-4), with higher BCS values associated with progressively higher odds of conception, regardless of the synchronization protocol (Figure 2).

Effects of cow body weight on conception

The variation of conception probability with cow body weight for each synchronization protocol is presented (Figure 3). For each synchronization protocol, conception probability increased gradually with increasing body weight. From the logistic model, body weight had a small but significant positive effect on odds of conception (OR=1.01; 95% CI: 1.00-1.02; p = 0.040). For every 10 kg increase in body weight, the odds of conception increased by 10%, indicating that heavier cows were more likely to conceive, regardless of the estrus and ovulation synchronization protocol used.

Discussion

Lack of significant variations in conception rate among synchronization protocols in the current study was consistent with the findings of a study that compared conception rates between 5-day and 7-day Co-synch + P_4ID protocols in suckled beef cows.[28] Pregnancy rates in Angus cattle were reported to be higher with the 5-day Co-synch + P_4ID protocol than

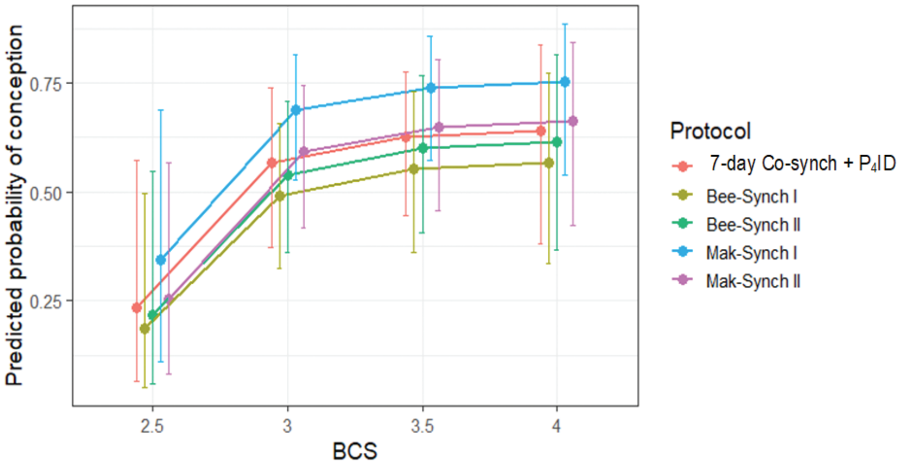

Figure 2. Variation of conception probability with BCS

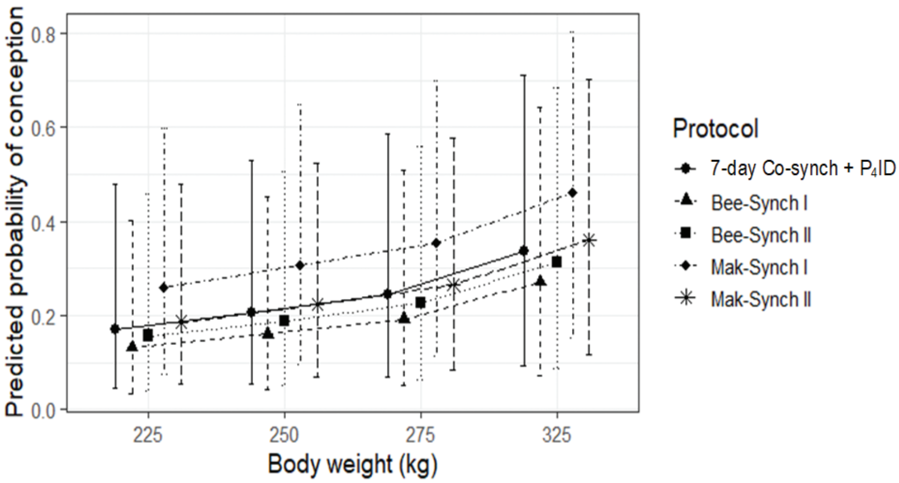

Figure 3. Variation of conception probability with body weight, stratified by treatment protocol

with the 7-day Co-synch + P_4ID,[13] or Bee synch I.[29] Differences in cattle genotype could be attributed to the reported variation of conception rate with synchronization protocol in these studies that was in purebred *B. taurus* cattle compared to the purebred *B. indicus* considered in the current study.

In a study with *B. indicus* cows, pregnancy rate obtained using the 5-day Co-synch + P_4ID, a related protocol to Bee synch I, was 36%. This was similar to the 37.26% obtained for Bee synch I protocol in the present study.[30] However, a recent study by the same group of researchers reported a pregnancy rate of 52% with the Bee synch I protocol[15] that was higher than the 37.26% obtained in our study. However, it is noteworthy that their research involved *B. taurus* x *B. indicus* crosses rather than pure *B. indicus* cows. Low conception rates obtained with the Bee synch I protocol in the current study were attributed to poor ovulation rate and ovulation synchronization due to asynchrony of follicular waves. First GnRH dose on day 0 (GnRH-1) of the Bee synch I protocol caused poor synchrony of follicular waves and ovulatory follicle size.[14] An alternative explanation for the low conception rate recorded under the Bee synch I protocol in the current study could be the failure of complete luteolysis of immature corpora lutea in the cows on day 5 of the treatments. This is possible because in the current study, the 2 doses of $PGF_{2\alpha}$ were given at once, at P_4ID withdrawal. However, 2 separate doses of $PGF_{2\alpha}$ given at an interval of 8-24 hours starting at P_4ID withdrawal are known to be required to ensure complete luteolysis of the corpus luteum in 5-day Co-synch + P_4ID protocols.[16,31,32] Therefore, future studies of Bee synch I protocol performance in *B. indicus* cows should consider the recommended interval of 8-24 hours between 2 $PGF_{2\alpha}$ treatments.

We hypothesized that increasing TAI and the last GnRH treatment improved conception rates by providing adequate time for additional growth of the dominant follicle before its stimulation to ovulate by the last exogenous GnRH. This was based on the notion that cows with large preovulatory follicles are more likely to ovulate, and produce high concentrations of estradiol, and subsequently progesterone, with positive effects on the uterine environment and embryo survivability.[33,34] However, no significant differences in conception rate were obtained among synchronization protocols. Lack of significant improvement in conception rate in the Mak Synch protocols compared to their original protocols could imply ineffectiveness of the current Mak Synch modifications.

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The basis for protocol modification was the prolonged ovulation interval encountered in SHZ cows synchronized with 7-day Co-synch + P_4ID and Bee Synch II protocols that involved GnRH-2 treatment concurrent with TAI.[17] Since the current Mak Synch modifications did not enhance fertility, it is likely that breed-specific intrafollicular characteristics were responsible for the protracted interval from luteinizing hormone (LH) surge to ovulation reported in the earlier study. Therefore, a study to determine the efficacy of extending only TAI while maintaining the original timing of GnRH-2 treatment at 64 hours after P_4ID withdrawal is recommended.

Another possible explanation for the absence of significant differences in conception rates among the tested synchronization protocols was the likely influence of uncontrolled biological variation among cows, especially their unknown days postpartum and cyclicity status at the onset of synchronization treatments. These variables are well-established determinants of fertility and responsiveness to hormonal synchronization protocols. Days postpartum is known to negatively affect uterine involution, resumption of ovarian cyclicity and endocrine function.[35] The negative effect of anestrus status of cows on pregnancy rate of cows bred after estrus and ovulation synchronization is also well documented.[36-38] Likewise, the estrous cycle phase at treatment onset determines the developmental status of ovarian follicles, the corpus luteum, and plasma progesterone concentrations before ovulation all of which influence fertility.[39-41] Consequently, cows entering the study while in anestrus, at various postpartum stages, or at different phases of the estrous cycle may have responded differently to identical synchronization treatments. Therefore, lack of difference in conception rate among the synchronization protocols should be viewed in the context of the likelihood of inherent variability introduced by unmeasured factors, particularly days postpartum, cyclicity status and estrous cycle phase at treatment onset. These factors may have introduced statistical noise, reducing the sensitivity of the analysis to detect true protocol-specific effects. Future studies that control for these variables are warranted to establish with certainty, the relative efficacy of the tested protocols in SHZ cows.

A methodological limitation of this study is that pregnancy diagnosis was conducted at 1 time point, 32 days after insemination, without subsequent confirmation of pregnancy retention at later stages of pregnancy (e.g. 60 and 90 days) or calving outcome. This is consequential because effects of synchronization protocol type on embryonic and fetal losses between 30 and 90 days of pregnancy are well-documented.[42-44] The quality and function of the CL are critical for progesterone production and pregnancy maintenance, may differ among synchronization protocols. Variations in CL vascularity, size, and progesterone output could predispose pregnancies established after certain protocols to subsequent loss, thereby obscuring true differences in protocol efficacy when only early conception is assessed. Therefore, our findings on day 32 reflected only early pregnancy establishment, not long-term survival. Future studies should incorporate sequential pregnancy status, including on days 60 and 90, or calving outcomes, to provide a more comprehensive evaluation of protocol success.

In the present study, cow nutritional condition, as represented by BCS and body weight, emerged as a strong predictor of conception following estrus synchronization and timed AI. These findings demonstrated a clear positive association between cow nutritional condition and conception rate. Our results were consistent with earlier reports that cows in better nutritional condition at insemination have higher pregnancy rates.[45-47] In particular, cows with a BCS > 2.75 on a scale of 1-5, were reported to have significantly higher pregnancy rates than thinner cows.[37] This pattern was also reflected in the present study, where cows with BCS ≥ 3.0 had higher conception rates than those with lesser scores. It has been reported that elongation of the P_4ID duration could enhance pregnancy rates in lactating *B. indicus* cows that are in low BCS.[48] Future studies should validate this hypothesis in SHZ cattle. The association between cow nutritional condition and fertility can be explained by the role of body energy reserves in reproductive physiology. Body condition score is an indicator of body fat and energy reserves,[49] and adequate fat reserves are crucial for ovarian cyclicity. In cows with poor BCS, the hypothalamus remains highly sensitive to estradiol's negative feedback, suppressing GnRH pulse generator and leading to infrequent LH pulses. This ultimately results in prolonged postpartum anestrus and anovulation.[50] On the contrary, cows in good body condition exhibit improved hypothalamic-pituitary responsiveness, facilitating earlier return to cyclicity. Additionally, adipose tissues are a primary site for leptin synthesis; there is a positive correlation between leptin concentrations and frequency of LH pulses.[51-53] Increased pulse frequency is essential for the maturation of the dominant follicle, expression of estrous behavior, and formation of a functional corpus luteum. Consequently, cows in superior nutritional condition are more likely to be cycling at the start of a synchronization protocol.[54] Therefore, ensuring breeding cows are in optimal nutritional condition is an important determinant of success in cattle bred after estrus and ovulation synchronization.

Conclusion

Reducing P_4ID implant duration from 7 to 5 days in Co-synch + P_4ID protocols, with additional doses of $PGF_{2\alpha}$ (1 at protocol onset and the other at P_4ID withdrawal) or without did not result in significant differences in conception rates in SHZ cows. Similarly, delaying the TAI, from 60-66 to 74-78 hours in 7-day Co-synch + P_4ID, or 64-68 to 74-78 hours in Bee synch II protocol, did not result in significant differences in conception rates in SHZ cows. More studies on breed-specific synchronization strategies are recommended with the ultimate goal of improving reproductive performance in *B. indicus* cattle. From a practical perspective, our findings emphasized that protocol choice alone is insufficient to guarantee success; optimizing cow nutrition and body reserves are a cornerstone of successful application of assisted reproductive technologies in cattle.

Ethics approval

Study was approved by the Institutional Animal Care and Use committee (IACUC) of the School of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, approval number #SVAR-IACIJC I98I2O2I, and by the Uganda National Council for Science and Technology (UNCST), approval number A219ES.

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Conflict of interest

Authors declare no competing interests.

Authors' contributions and declaration

PM, **SK**, **CL**, **MN** and **JO** contributed to the experiment design and methods. **PM** and **SK** implemented experiments and collected data. **PM**, **MN** and **JO** interpreted analyzed data. **PM** drafted the initial manuscript; **MN**, **CL**, **DO**, **DM**, **BK** and **JO** edited the draft. All authors have read and approved the final version of the manuscript.

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