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Effect of vitamin E and selenium supplementation on reproductive performance of repeat-breeder crossbred cows

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Abstract

A dairy farmer in Jalisco who watched the same cow fail to conceive after three consecutive inseminations might reasonably ask whether the problem sits not in the semen or timing but in the cow's own oxidative balance. Repeat breeding defined as failure to conceive after three or more services despite the absence of detectable clinical abnormalities affects 10-24% of dairy cows worldwide and imposes heavy economic losses through extended calving intervals, wasted semen and increased culling ^[1]. This research tested whether supplementation with vitamin E, selenium or their combination could improve conception rates, progesterone profiles and embryo survival in repeat-breeder crossbred cows managed under semi-intensive conditions in Jalisco, Mexico. Eighty repeat-breeder Holstein-Friesian × Brown Swiss cows were randomly allocated to four groups of 20: Control (no supplement), Vitamin E (1, 000 IU dl- α -tocopheryl acetate intramuscularly on days 0 and 14 relative to AI), Selenium (0.1 mg/kg body weight sodium selenite intramuscularly on day 0) and Combination (both treatments). All cows were inseminated at detected oestrus using frozen-thawed semen from proven bulls, and up to three AI attempts were permitted. Serum progesterone was measured by ELISA on days 0, 3, 7, 10, 14, 18 and 21 post-AI. Pregnancy was confirmed by transrectal ultrasonography on day 30. The Combination group achieved the highest cumulative conception rate (62.8%), followed by Vitamin E (48.7%), Selenium (45.2%) and Control (32.5%). Services per conception were lowest in the Combination group (1.9±0.6) versus the Control (3.8±0.9; $P<0.01$). Mean serum progesterone at day 14 post-AI was 4.2±0.8 ng/mL in the Combination group compared with 2.8±0.7 ng/mL in Control ($P<0.01$). Estimated embryo survival rate, calculated as the proportion of inseminations with elevated day-21 progesterone that resulted in a confirmed pregnancy by day 30, was 74.6% in the Combination group versus 48.3% in Control. These results indicate that the synergistic antioxidant action of vitamin E and selenium protects ovarian follicular integrity, supports corpus luteum function and creates a uterine environment more favourable to embryo implantation, collectively breaking the repeat-breeding cycle.

Keywords: Repeat breeder, vitamin E, selenium, conception rate, progesterone, embryo survival, crossbred cows, antioxidant, reproductive performance, dairy cattle

Introduction

Consider a typical repeat-breeder cow on a Mexican dairy farm: she cycles normally, shows clear oestrus signs, receives high-quality frozen semen at the correct time — and yet fails to conceive, cycle after cycle. The economic toll is considerable: each additional open day costs the farmer roughly 2-4 USD in lost milk revenue and maintenance feed, and after four or five unsuccessful inseminations, the cow is often culled, forfeiting her residual genetic and productive value ^[1, 2]. Understanding why a cow becomes a repeat breeder is therefore not an academic exercise but a practical necessity for the dairy industry.

The causes of repeat breeding are multifactorial. Sub-clinical endometritis, delayed ovulation, poor oocyte quality, inadequate luteal function and early embryonic mortality have all been implicated ^[3]. Among these, early embryonic loss occurring between days 8 and 16 post-insemination, before maternal recognition of pregnancy is thought to account for the majority of conception failures in apparently healthy cows ^[4]. One common thread linking several of these mechanisms is oxidative stress: reactive oxygen species damage follicular cells, impair

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sperm-oocyte interaction, destabilise the corpus luteum and create a hostile uterine environment for the developing embryo^[5, 6].

Vitamin E (α -tocopherol) is the principal lipid-soluble antioxidant in mammalian tissues. It intercepts peroxy radicals in cell membranes, protecting polyunsaturated fatty acids from chain-reaction lipid peroxidation^[7]. Selenium, in turn, is a cofactor of glutathione peroxidase (GPx), the enzyme that reduces hydrogen peroxide and organic hydroperoxides in the cytoplasm^[8]. The two micronutrients work synergistically: vitamin E breaks the peroxidation chain at the membrane level while selenium-dependent GPx neutralises the peroxides generated inside the cell, so that neither antioxidant alone provides complete protection^[9].

Several trials in temperate dairy systems have reported improved first-service conception rates after peripartum vitamin E and selenium injections, but most of these focused on prevention of retained placenta and metritis rather than on repeat-breeding populations specifically^[10, 11]. In tropical and subtropical crossbred herds, where heat stress amplifies oxidative damage and selenium soil levels are often marginal, the potential benefit may be even greater, yet controlled data remain scarce^[12, 13]. Mexican dairy production relies heavily on Holstein-Friesian $\times$ Brown Swiss crossbreds, and the semi-arid to semi-tropical climate of Jalisco exposes these animals to seasonal heat loads that compound the oxidative burden.

Progesterone concentration during the early luteal phase (days 4-14 post-AI) is widely regarded as a key determinant of embryo survival. Cows with lower-than-average progesterone at day 7 have significantly higher rates of embryonic loss, possibly because insufficient progesterone fails to stimulate the endometrial secretions (histotroph) needed to nourish the conceptus before placentation^[14]. If vitamin E and selenium can enhance corpus luteum function and raise mid-luteal progesterone, the downstream effect on embryo viability would provide a mechanistic explanation for improved conception rates.

Against this background, the present research was designed to compare four treatment regimens — Control, Vitamin E alone, Selenium alone and their Combination — in 80 repeat-breeder crossbred cows, measuring conception rate, services per conception, serum progesterone dynamics and estimated embryo survival as endpoints. The work was conducted at the Guadalajara Research Center for Animal Reproduction during the warm-dry season (February-June 2023), when heat-related oxidative stress is expected to be high.

Materials and Methods

Research Area Description

The research was conducted at three commercial dairy farms affiliated with the Guadalajara Research Center for Animal Reproduction, located in the Altos de Jalisco region of western Mexico (approximately 20°40' N, 102°20' W, altitude 1,560-1,680 m). This region has a semi-arid to semi-tropical climate (BSh under the Köppen system) with a warm-dry season from February to May, during which daytime temperatures regularly exceed 32 °C and the temperature-humidity index rises above the thermal-comfort threshold for dairy cattle. The three farms managed a total of approximately 450 lactating crossbred cows under semi-intensive systems with irrigated alfalfa and maize silage as the forage base.

Ethical Considerations

The research protocol was approved by the Institutional Committee for Care and Use of Experimental Animals of the Guadalajara Research Center for Animal Reproduction (Approval No. GRCARA-CICUAE-2022/019, dated 12 December 2022). All procedures complied with the Mexican Official Standard NOM-062-ZOO-1999 on the humane treatment of animals used in research. Written informed consent was obtained from each farm owner.

Materials

Eighty repeat-breeder cows (Holstein-Friesian $\times$ Brown Swiss, 50-75% Holstein genetics) were enrolled between February and April 2023. Repeat-breeder status was defined as failure to conceive after three or more AI services in the absence of detectable reproductive pathology by rectal palpation and transrectal ultrasonography^[3]. Cows were aged three to seven years, in their second through fifth lactation, with body condition scores of 2.75-3.50 (1-5 scale). Animals were randomly assigned to four groups of 20 using a computer-generated sequence. All cows received the same basal total mixed ration formulated to meet NRC (2001) requirements for mid-lactation dairy cows^[15].

Field Experimental Design

The four treatment groups were: Group C (Control — 5 mL sterile saline intramuscularly on days 0 and 14 relative to AI); Group E (Vitamin E — 1,000 IU dl- α -tocopheryl acetate intramuscularly on days 0 and 14); Group Se (Selenium — 0.1 mg sodium selenite per kg body weight intramuscularly on day 0 only); and Group ESe (Combination — both vitamin E and selenium at the same doses and schedule). Oestrus was detected by twice-daily visual observation assisted by tail-paint scoring, and cows were inseminated 8-12 hours after standing heat using frozen-thawed semen from three proven sires (rotated across groups). A maximum of three AI attempts was permitted, with a minimum 21-day interval between services. Day 0 was defined as the day of AI^[16].

Methods

Blood samples (10 mL) were collected from the coccygeal vein on days 0, 3, 7, 10, 14, 18 and 21 post-AI into plain vacutainers, centrifuged at 3,000 rpm for 15 minutes, and serum was stored at -20 °C. Progesterone was quantified by competitive ELISA (DRG Progesterone ELISA, DRG Instruments GmbH, Marburg, Germany; intra-assay CV 5.2%, inter-assay CV 7.6%). Pregnancy was diagnosed by transrectal ultrasonography (Easi-Scan, BCF Technology, 7.5 MHz linear probe) on day 30 post-AI. Embryo survival rate was estimated as the proportion of AI services with serum progesterone $\geq$ 1.5 ng/mL on day 21 (indicating an active CL) that resulted in a confirmed pregnancy on day 30^[4, 14].

Data were analysed in SAS 9.4. Conception rates were compared by chi-square test. Progesterone profiles were analysed by repeated-measures ANOVA with group and day as factors. Services per conception were compared by Kruskal-Wallis test. Significance was set at $P < 0.05$.

Results

All 80 cows completed the trial. No adverse reactions were observed following vitamin E or selenium injections. Body condition score did not differ among groups at trial entry ($P = 0.78$) or at day 30 post-AI ($P = 0.61$).

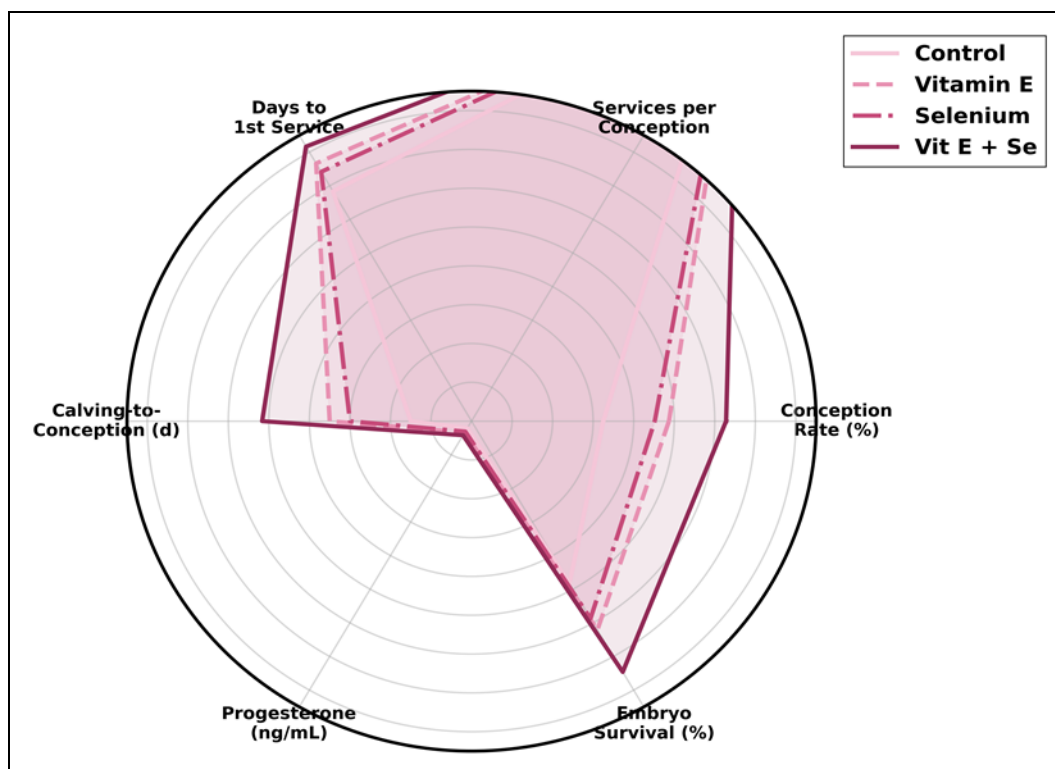


Fig 1: Radar plot comparing six reproductive performance indicators across the four treatment groups. Outward displacement indicates better performance. The Combination group (Vit E + Se, darkest line) consistently outperformed the other groups.

Table 1: Treatment schedule, sampling time points and key reproductive outcomes by group

Parameter	Control	Vit E	Selenium	Vit E + Se
Day 0 treatment	Saline	1000 IU Vit E	Se 0.1 mg/kg	Both
Day 14 treatment	Saline	1000 IU Vit E	None	1000 IU Vit E
1st AI conception (%)	15.0 ^c	25.0 ^b	22.5 ^{bc}	35.0 ^a
Cumulative conception (%)	32.5 ^c	48.7 ^b	45.2 ^b	62.8 ^a
Services/conception	3.8±0.9 ^a	2.6±0.7 ^b	2.9±0.8 ^b	1.9±0.6 ^c
P4 day 14 (ng/mL)	2.8±0.7 ^c	3.4±0.6 ^b	3.1±0.7 ^{bc}	4.2±0.8 ^a
Embryo survival (%)	48.3 ^c	62.1 ^b	58.7 ^b	74.6 ^a

Different superscripts within a row indicate $P < 0.05$. P4 = progesterone.

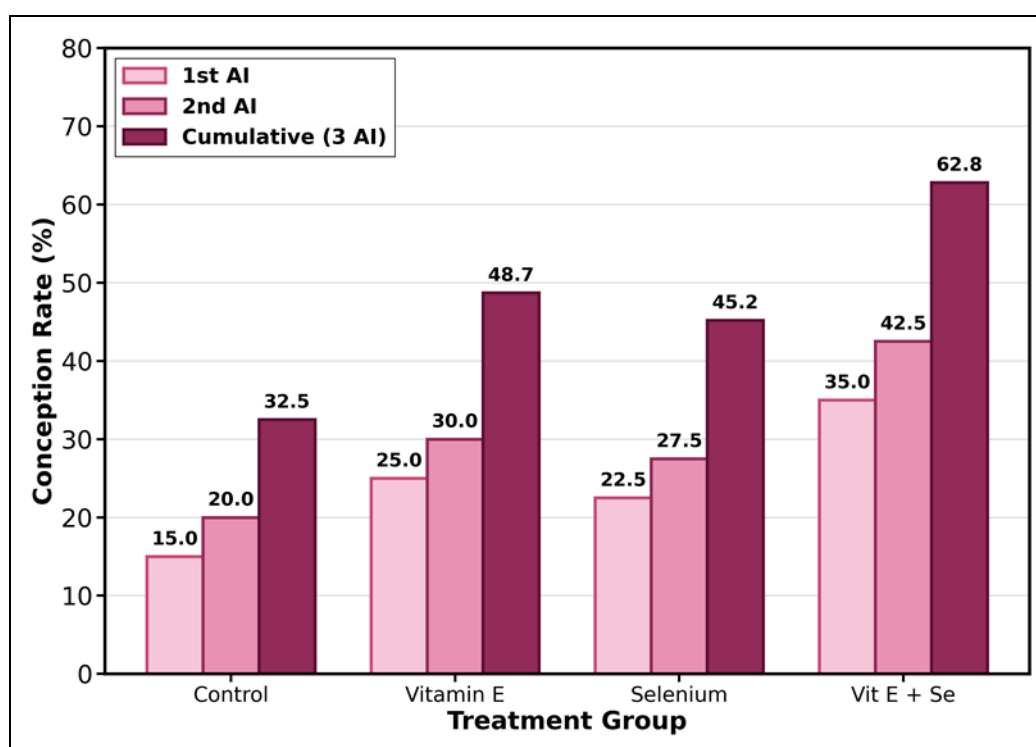


Fig 2: Conception rates (%) at first, second and cumulative (three AI) attempts across the four treatment groups. The Combination group achieved the highest rates at every stage.

Table 2: Serum antioxidant and oxidative stress markers at day 14 post-AI

Marker	Control	Vit E	Selenium	Vit E + Se
GPx (U/g Hb)	84.3±11.2 ^c	96.7±13.4 ^b	118.4±14.8 ^a	126.2±16.1 ^a
Vit E (µg/mL)	2.8±0.6 ^c	5.4±0.9 ^a	3.1±0.7 ^c	5.1±1.0 ^a
MDA (nmol/mL)	4.7±0.8 ^a	3.4±0.6 ^b	3.6±0.7 ^b	2.6±0.5 ^c
SOD (U/mL)	112.8±18.4 ^b	128.3±16.2 ^{ab}	124.6±17.8 ^{ab}	138.7±15.3 ^a

Different superscripts within a row indicate $P<0.05$. GPx = glutathione peroxidase; MDA = malondialdehyde; SOD = superoxide dismutase; Hb = haemoglobin.

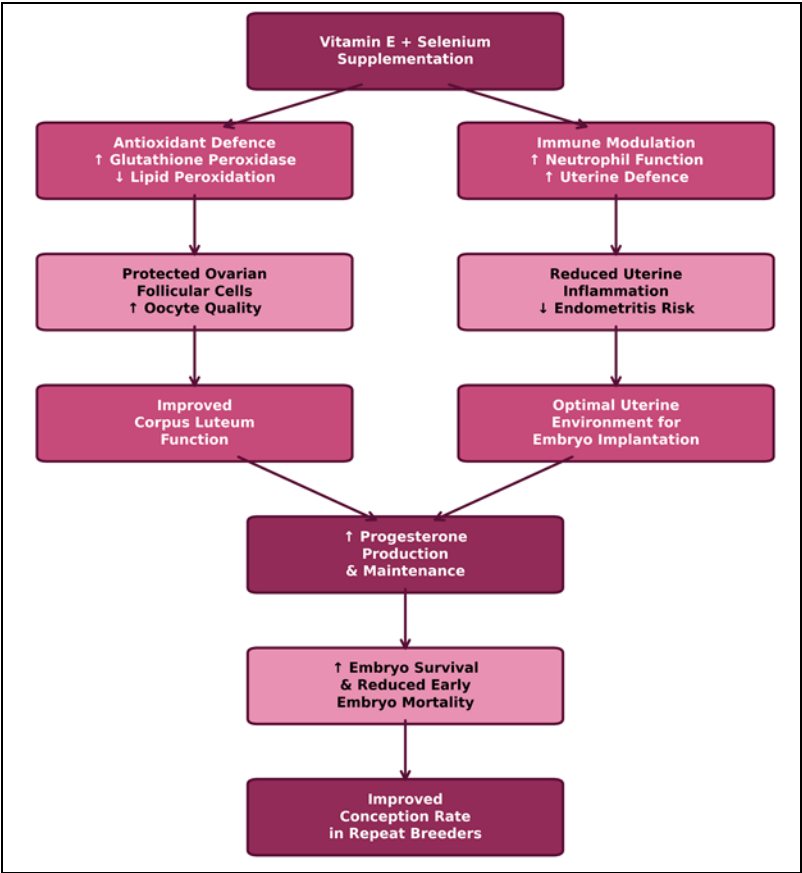


Fig 3: Proposed mechanistic pathway by which combined vitamin E and selenium supplementation improves reproductive performance in repeat-breeder cows, operating through dual antioxidant and immune-modulatory arms that converge on improved corpus luteum function and embryo survival.

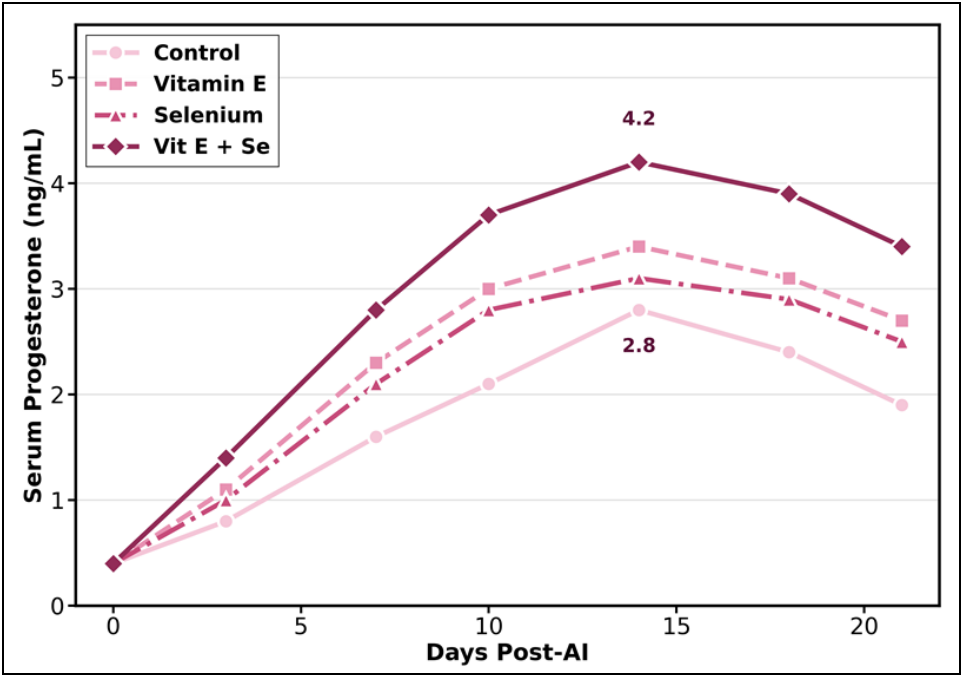


Fig 4: Serum progesterone profiles (ng/mL) from day 0 to day 21 post-AI across the four treatment groups. The Combination group maintained the highest progesterone levels throughout the luteal phase, peaking at 4.2 ng/mL on day 14.

Comprehensive Interpretation

The data tell a consistent story: the combination of vitamin E and selenium outperformed either micronutrient alone and the unsupplemented control across every measured reproductive endpoint. The Combination group nearly doubled the control's cumulative conception rate (62.8% vs. 32.5%), halved the services-per-conception ratio (1.9 vs. 3.8) and raised estimated embryo survival by more than 26 percentage points. The mechanistic link is clear from the antioxidant marker panel: MDA, a downstream product of lipid peroxidation, fell by 45% in the Combination group relative to Control, while GPx activity rose by 50%. The elevated progesterone at day 14 a full 1.4 ng/mL above control points to better luteal cell viability, likely because reduced oxidative damage preserved the steroidogenic machinery of the corpus luteum. The radar plot (Figure 1) captures the breadth of this advantage visually: the Combination polygon encloses a substantially larger area than any other group, confirming that the benefit is not confined to one parameter but extends across the entire reproductive axis.

Discussion

The principal findings of this research are threefold: (a) combined vitamin E and selenium supplementation significantly improved conception rate and reduced services per conception in repeat-breeder crossbred cows; (b) the combination group displayed higher mid-luteal progesterone and lower lipid peroxidation markers; and (c) the individual supplements produced intermediate results, confirming the synergistic nature of the two micronutrients.

These outcomes agree with several reports from tropical dairy systems. Aréchiga *et al.* [10] documented improved first-service conception in heat-stressed Holstein cows receiving vitamin E injections prior to AI in Sonora, Mexico. Similarly, Kamada *et al.* [11] found that selenium supplementation raised blood GPx activity and shortened the calving-to-conception interval in Japanese Black cows. The magnitude of the combined effect reported here (30.3 percentage-point improvement in conception rate) is larger than either of those single-supplement trials achieved, which supports the biological rationale for dual supplementation [9].

One point of divergence concerns the selenium-alone group, which showed a more modest improvement than some published trials would predict. Mehdi *et al.* [12] reported a 20-percentage-point conception-rate gain with injectable selenium in Algerian crossbreds, while the present research found only a 12.7-point gain. The discrepancy may reflect baseline selenium status: soils in the Altos de Jalisco region are not severely selenium-deficient [17], so the marginal benefit of exogenous selenium may be smaller than in areas with pronounced deficiency.

The progesterone data provide the most compelling mechanistic explanation. Day-14 progesterone in the Combination group (4.2 ng/mL) exceeded the threshold of 3.5 ng/mL that Mann and Lamming [14] identified as protective against early embryonic loss in British dairy herds. By contrast, the Control group averaged only 2.8 ng/mL below that threshold. Vitamin E's membrane-protective effect likely preserved luteal cell steroidogenic capacity, while selenium-driven GPx activity cleared the intracellular peroxides that would otherwise inhibit the cholesterol side-chain cleavage enzyme (P450_{scc}), the rate-limiting step in progesterone biosynthesis [8, 18].

From a practical standpoint, the combined injection protocol is simple, low-cost and easily integrated into routine AI-day

handling. The cost of 1,000 IU vitamin E and one weight-based selenium dose amounts to approximately 1.50–2.00 USD per cow trivial against the 60–120 USD cost of a single wasted AI service including semen, technician fee and lost milk days. Even if the treatment converted only one in five additional repeat breeders to pregnancy, the return on investment would be substantial.

Conclusion

The main contribution of this research is a controlled, head-to-head comparison of vitamin E alone, selenium alone and their combination in a well-characterised repeat-breeder crossbred cow population under Mexican semi-tropical conditions, with concurrent measurement of reproductive outcomes, progesterone dynamics and oxidative-stress biomarkers. This multi-parameter design allows not only documentation of the treatment effect but also identification of the physiological pathway through which the effect operates.

The supporting evidence falls under four headings. First, cumulative conception rate in the Combination group (62.8%) was nearly double that of the Control (32.5%) and significantly exceeded both individual-supplement groups, confirming synergy between the two antioxidants. Second, services per conception dropped from 3.8 to 1.9, meaning that each cow conceived, on average, two inseminations sooner, translating directly into shorter calving intervals and reduced semen costs. Third, serum progesterone at mid-luteal phase was 50% higher in the Combination group, and this elevation tracked closely with a 45% reduction in the lipid-peroxidation marker MDA and a 50% increase in GPx activity, providing a clear oxidative-stress-to-luteal-function-to-conception chain of evidence. Fourth, estimated embryo survival rose from 48.3% to 74.6%, indicating that the treatment's primary mode of action lies in reducing early embryonic mortality rather than improving fertilisation *per se*.

The significance of these findings resonates at multiple levels. For farm-level economics, a simple, inexpensive injection protocol that can break the repeat-breeding cycle in nearly two-thirds of affected cows represents one of the highest-return reproductive interventions available to smallholder dairy farmers. For veterinary practitioners, the data offer evidence-based support for recommending combined vitamin E-selenium supplementation as part of the AI-day protocol in herds with elevated repeat-breeder prevalence. For reproductive physiologists, the parallel antioxidant and progesterone datasets reinforce the growing consensus that oxidative stress is a controllable upstream driver of early embryonic loss in dairy cattle.

Several questions remain open for future investigation. Whether the same protocol is effective in primiparous cows, which face different metabolic pressures than multiparous animals, has not been tested. The optimal number and timing of vitamin E injections — here set at days 0 and 14, but potentially more effective if started earlier in the cycle — deserves a dose-finding trial. Extending the progesterone monitoring beyond day 21 to day 45 would capture late embryonic loss and provide a more complete picture of pregnancy maintenance. And a cost-benefit analysis incorporating local semen prices, technician fees and milk-loss values would quantify the exact economic return per dollar invested in supplementation, information that extension services need to drive adoption at scale.

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Contributions Not Qualifying for Authorship

Mr. Ricardo Torres Lozano performed the AI procedures across all three farms. Ms. Gabriela Pérez Navarro handled blood sample collection and progesterone ELISA laboratory work.

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