INPLASY

Second prostaglandin $F_2\alpha$ dose in dairy cows with Ovsynch protocols with or without progesterone: systematic review and meta-analysis of luteolysis, pregnancy, and pregnancy loss

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Espinoza-Villavicencio, JL; Murillo-Amador, B; Ortega-Perez, R.

Corresponding author:
Jose Luis Espinoza Villavicencio

jlvilla@uabcs.mx

Author Affiliation:
UNIVERSIDAD AUTONOMA DE BAJA CALIFORNIA SUR.

ADMINISTRATIVE INFORMATION

Support - The study received no specific funding from public, commercial, or private-sector agencies.

Review Stage at time of this submission - Completed but not published.

Conflicts of interest - None declared.

INPLASY registration number: INPLASY202680047

Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 11 August 2026 and was last updated on 11 August 2026.

INTRODUCTION

Review question / Objective Primary question: Does the addition of a second prostaglandin $F_2\alpha$ ($PGF_2\alpha$) injection to a fixed-time artificial insemination (FTAI) protocol improve the pregnancy per artificial insemination (P/AI) rate in dairy cattle compared to protocols using a single $PGF_2\alpha$ injection?
Objective:
To systematically identify, appraise, and meta-analyze all available randomized evidence comparing double-dose versus single-dose $PGF_2\alpha$ in dairy cattle FTAI protocols, and to estimate the pooled effect on P/AI with assessment of heterogeneity and certainty of evidence.

Rationale Fixed-time artificial insemination (FTAI) protocols in dairy cattle rely on exogenous prostaglandin $F_2\alpha$ ($PGF_2\alpha$) to ensure complete luteolysis and appropriate synchronization of follicular and luteal dynamics. The standard Ovsynch protocol and its variants typically include one $PGF_2\alpha$ injection; however, incomplete luteolysis following a single dose has been identified as a source of synchronization failure, particularly in cows with older or more resistant corpora lutea. An additional (second) $PGF_2\alpha$ injection, administered approximately 24 hours after the first, has been proposed to improve complete luteolysis and, consequently, synchronization efficiency and conception rates.
Despite the theoretical rationale, individual published studies have yielded inconsistent results, likely due to differences in protocol design, breed, parity, lactation status, progesterone supplementation, and sample size. A systematic review and meta-analysis is therefore necessary to pool the available randomized evidence, reduce uncertainty, and provide a robust quantitative estimate of the effect of a second $PGF_2\alpha$ injection on P/AI. To date, no registered systematic review has addressed this specific question in dairy cattle.

Condition being studied Reproductive efficiency in dairy cattle, specifically defined as the

probability of achieving pregnancy following a single fixed-time AI event. Low P/AI rates are a primary driver of extended calving intervals, elevated culling rates, and reduced economic productivity in dairy herds worldwide. Optimizing FTAI protocols — including the number of $PGF_2\alpha$ doses administered — is a key strategy to improve herd reproductive performance. This review focuses specifically on the role of $PGF_2\alpha$ double-dosing within FTAI synchronization programs.

METHODS

Search strategy A documented, structured search strategy developed in accordance with the PRISMA-S guidelines (Rethlefsen et al., 2021) was conducted on May 8, 2026, in PubMed/MEDLINE, Dimensions, Consensus, Web of Science Core Collection, Scopus, ScienceDirect, and Wiley Online Library. A targeted update was conducted through June 22, 2026, using reference-list screening, citation tracking, and searches of institutional repositories. No language restrictions were applied to the final selection.

Participant or population Dairy cows (Bos taurus, Bos indicus, and crossbreeds) enrolled in any variant of a fixed-time artificial insemination (FTAI) synchronization protocol. Eligible populations include:
• Lactating cows and non-lactating cows in any stage of the postpartum period
• Primiparous and multiparous cows
• Any breed (Holstein-Friesian, Jersey, Simmental, Zebu-crossbreeds, etc.)
• Cows managed in any production system (tie-stall, free-stall, pasture-based)
• Any country or climatic region
Exclusion: Studies conducted exclusively in beef cattle (non-dairy breeds managed solely for meat production) were excluded, as reproductive physiology, management conditions, and outcome benchmarks differ substantially. Studies in ewes, mares, or other species were also excluded.

Intervention Administration of two doses of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) within a fixed-time AI synchronization protocol, where the second dose is given approximately 24 hours after the first $PGF_2\alpha$ injection. The intervention encompasses:
• Any synthetic or natural $PGF_2\alpha$ analogue (cloprostenol, dinoprost tromethamine, luprostiol, d-cloprostenol)
• Any double-dose $PGF_2\alpha$ schedule within Ovsynch-based, progesterone-based, or other FTAI variants
• Any route of administration (intramuscular, subcutaneous)
• Any dose per injection consistent with manufacturer label recommendations
Sub-protocols were classified a priori into two categories based on concurrent use of exogenous progesterone devices: (a) protocols without exogenous progesterone (e.g., standard Ovsynch, Double-Ovsynch, G6G) and (b) protocols with exogenous progesterone (e.g., PRID, CIDR).

Comparator Fixed-time AI protocols using a single dose of $PGF_2\alpha$, administered according to the standard schedule for each protocol type (e.g., single $PGF_2\alpha$ on Day 7 of Ovsynch). The comparator must be concurrent (within the same study/trial arm), not historical. Studies comparing a single-dose FTAI protocol to non-FTAI management were excluded.

Study designs to be included Randomized controlled experiments (RCEs), including:• Parallel-group randomized field trials• Cross-over designs with appropriate washout periods (if applicable)• Cluster-randomized trials where the unit of randomization is the herd or pen (with appropriate statistical adjustment)Non-randomized studies, observational studies, case series, and simulation studies were excluded. Studies described as "randomized" were assessed for randomization integrity using the Cochrane Risk of Bias tool version 2 (RoB 2).

Eligibility criteria Inclusion criteria:
1. Dairy cattle (see Item 12)
2. Intervention: FTAI protocol with double-dose $PGF_2\alpha$ (see Item 13)
3. Comparator: FTAI protocol with single-dose $PGF_2\alpha$, concurrent (see Item 14)
4. Outcome: Pregnancy/conception diagnosis per AI event as a binary outcome
5. Study design: Randomized controlled experiment (see Item 15)
6. Unit of randomization: individual cow, pen, or herd
Exclusion criteria:
1. Studies exclusively in beef cattle or other species
2. Non-randomized designs
3. Studies not reporting P/AI or an equivalent binary pregnancy outcome
4. Arms that differ in any protocol component other than the number of $PGF_2\alpha$ injections
5. Conference abstracts without sufficient data for RoB assessment and effect extraction (unless raw data obtainable from authors)
6. Duplicate publications of the same dataset (only the most complete report retained)
No language restriction was applied during search or screening.

Information sources PubMed/MEDLINE, Dimensions, Consensus, Web of Science Core Collection, Scopus, ScienceDirect, and Wiley OnlineLibrary, Reference lists of included studies, Reference lists of relevant reviews.

Main outcome(s) Pregnancy per artificial insemination (P/AI)

• Definition: Binary outcome — the cow was diagnosed as pregnant (1) or not pregnant (0) at a minimum of 28 days after the fixed-time AI event, via transrectal ultrasonography, progesterone assay, or similar validated method.

• Effect measure: Risk Ratio (RR) with 95% confidence intervals (CI)

• Timing: Assessment at first pregnancy diagnosis post-AI (≥28 days)

• Justification: P/AI is the standard primary reproductive efficiency indicator in dairy cattle research and is consistently reported across studies in this area.

Additional outcome(s) 1. Subgroup effect by exogenous progesterone use: Comparison of double-dose vs. single-dose $PGF_2\alpha$ effect within protocols with and without concurrent progesterone device (CIDR/PRID).

2. Sensitivity analysis outcome: Stability of the pooled RR after excluding studies classified as "some concerns" in Domain 1 (randomization process) of RoB 2.

Data management Screening: Two independent reviewers screened titles/abstracts and subsequently full texts against the eligibility criteria. Disagreements at each stage were resolved by consensus; unresolved discrepancies were adjudicated by a third reviewer.

Data extraction: Two reviewers independently extracted data using a standardized extraction form including: first author, year, country, breed, parity, protocol type, $PGF_2\alpha$ analogue and dose, timing of second injection, use of exogenous progesterone, sample size per arm, number of pregnant cows per arm, and method of pregnancy diagnosis.

Software: RAYYAN QCRI.

Quality assessment / Risk of bias analysis Tool used: Cochrane Risk of Bias tool, version 2 (RoB 2) for randomized trials.

RoB 2 assesses five domains:

• Domain 1: Bias arising from the randomization process

• Domain 2: Bias due to deviations from intended interventions

• Domain 3: Bias due to missing outcome data

• Domain 4: Bias in measurement of the outcome

• Domain 5: Bias in selection of the reported result

Each study received an overall RoB 2 judgment of Low risk, Some concerns, or High risk. Studies with "High risk" in any domain were excluded from the primary analysis. Studies with "Some concerns" in Domain 1 were retained in the primary analysis but excluded in the pre-specified sensitivity analysis.

Certainty of evidence: The GRADE framework was applied to rate the certainty of the body of evidence for the primary outcome across five domains: risk of bias, inconsistency, indirectness, imprecision, and publication bias.

Strategy of data synthesis Statistical approach: Quantitative synthesis (meta-analysis) using a random-effects model with the DerSimonian–Laird estimator for the between-study variance (τ^2).

Effect measure: Risk Ratio (RR) with 95% CI for the binary outcome P/AI.

Heterogeneity assessment:

• τ^2: Between-study variance

• I^2: Proportion of total variability attributable to between-study heterogeneity; interpreted as: 50% substantial

• χ^2 test (Cochran's Q): Global test for heterogeneity

Threshold for meta-analysis: At least 3 eligible studies with extractable data for the primary outcome.

Metaregression: Not planned a priori nor performed due to the very low observed heterogeneity ($I^2 \leq 6\%$ in the primary analysis), which would render metaregression uninformative and statistically inappropriate.

Publication bias: Funnel plot inspection and Egger's test if ≥10 studies were available.

SOFTWARE: Python (versión 3.x; Python Software Foundation) using the SciPy and NumPy packages.

Subgroup analysis Pre-specified subgroup: Use of exogenous progesterone in the FTAI protocol.

• Subgroup A — Protocols without exogenous progesterone (e.g., Ovsynch, Double-Ovsynch, G6G)

• Subgroup B — Protocols with exogenous progesterone (e.g., Ovsynch + CIDR, J-Synch + PRID)

Rationale: Exogenous progesterone influences luteal function and synchronization outcomes independently of $PGF_2\alpha$; its presence may modify the marginal effect of a second $PGF_2\alpha$ dose.

Test for subgroup differences: χ^2 test on the between-subgroup contrast.

Sensitivity analysis Pre-specified sensitivity analysis: Exclusion of studies classified as "Some

concerns" in Domain 1 (bias arising from the randomization process) of RoB 2.
Studies excluded: Alnimer 2019, Alsuwaidawi 2024, Hölper 2023, Kuru 2020 (Ovsynch+CIDR arm), McDougall 2021 (progesterone subgroup), Rheinberger 2020, and Tippenhauer 2021.
Purpose: To assess whether the pooled estimate is robust to uncertainty in the adequacy of the randomization process among studies with incomplete reporting of allocation procedures.
Result: The sensitivity analysis (15 comparisons, 12 studies; 3,662 experimental vs. 3,415 control cows) yielded RR = 1.06 (95% CI: 1.00–1.13; p = 0.04; I^2 = 0%), confirming the robustness of the primary analysis.

Language restriction No language restrictions were applied during the database search or study selection process. Studies published in any language were eligible. Non-English papers were translated with the assistance of as needed.

Country(ies) involved Mexico — Universidad Autónoma de Baja California Sur (UABCS), La Paz, Baja California Sur.

Other relevant information 1. Retrospective registration: As noted in Item 4, this protocol is registered retrospectively. The review has been completed, and the manuscript has been prepared. Registration is undertaken to contribute to transparency in veterinary evidence synthesis and to comply with the recommendations of journals and evidence-synthesis bodies for all systematic reviews, regardless of species studied.
2. PROSPERO ineligibility: This systematic review was not registered in PROSPERO because that platform restricts registration to systematic reviews with a direct and obvious bearing on human health outcomes. INPLASY, which explicitly accepts systematic reviews of animal studies, is the appropriate registration platform.
3. PRISMA compliance: The review was conducted and reported in accordance with the PRISMA 2020 statement (Page et al., 2021) and PRISMA for Animals (where applicable).
4. Pre-specified metaregression waiver: Metaregression was not performed due to the very low between-study heterogeneity observed ($I^2 \leq$ 6% across all analyses), which is below the threshold at which metaregression provides meaningful additional explanatory power. This decision was made after observing the data and is disclosed transparently.

Keywords 'dairy cattle'; 'fixed-time artificial insemination'; 'prostaglandin F2alpha'; 'PGF2α double dose'; 'pregnancy per AI'; 'synchronization protocol'; 'meta-analysis'; 'systematic review'.

Dissemination plans The results of this systematic review and meta-analysis will be disseminated through:
1. Peer-reviewed journal publication in a journal specializing in animal reproduction, veterinary science, or livestock production (e.g., Theriogenology, Journal of Dairy Science, Animal Reproduction Science, Livestock Science).
2. Conference presentation at relevant national or international congresses in animal reproduction or veterinary science, if applicable.
3. Institutional dissemination through the Universidad Autónoma de Baja California Sur (UABCS) research channels.
The findings are intended to inform veterinary practitioners, reproductive management consultants, and dairy producers on the evidence base for double-dose $PGF_2\alpha$ protocols in FTAI programs.

Contributions of each author
Author 1 - JOSE LUIS ESPINOZA VILLAVICENCIO - Conceptualization, methodology, interpretation, and writing.
Email: jlvilla@uabcs.mx
Author 2 - RICARDO ORTEGA PEREZ - Conceptualization, methodology, interpretation, and writing.
Email: rortega@uabcs.mx
Author 3 - BERNARDO MURILLO AMADOR - Methodology, validation, formal analysis, manuscript review and editing, and correspondence.
Email: bmurillo04@cibnor.mx