



Fine-tuning folate and micronutrient profiles to reduce embryo resorption in domestic rabbits

Nazariy Fedynshyn
nazariyfedynshynedu@gmail.com



Zoogoods LLC, 123 Maple Ave., Suite 400, Springfield, IL 62704, USA

ORCID:

N. Fedynshyn <https://orcid.org/0009-0005-9096-0092>

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FN: Conceptualization; Investigation; Data curation; Formal analysis; Methodology; Writing — original draft, review & editing.

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Fine-tuning dietary folate and allied micronutrients may curb early embryo loss and boost litter success in domestic rabbits. This review asked which nutrient cocktails and doses actually work. *Web of Science*, *Scopus*, and *CAB Abstracts* were searched (1971–2024), 68 trials emerged. Two reviewers independently screened to pre-registered criteria, retaining 15 rigorously controlled studies. Effect sizes for embryo resorption and litter size were pooled with random-effects models, heterogeneity was explored by meta-regression. Folate at 3–5 mg kg⁻¹ feed cut resorption by 29 % (95 % CI 18–39). Adding 80 mg kg⁻¹ zinc lifted the effect by another 11 points, while selenium-vitamin E blends offered similar gains, suggesting oxidative and epigenetic synergy. Vitamin D₃ proved a double-edged sword-helpful below 3 000 IU kg⁻¹ yet growth-suppressive above. Heterogeneity sat at 62 %, driven mainly by dose spread and breed. Evidence favours a moderated multi-nutrient approach over single-factor fixes, but small samples and uneven protocols still cloud causality. Next-generation studies should weld metabolomics to adaptive feeding and turn nuanced optimisation into everyday practice.

Key words: domestic rabbits, fertility, embryo resorption, folate supplementation, zinc and selenium-vitamin E synergy, reproductive nutrition

Introduction

Embryo resorption—those silent, early losses that never reach the nest box—erodes both productivity and welfare in commercial rabbitries, yet it rarely makes the front page of nutrition manuals. Producers notice only that a promising pregnancy becomes a disappointingly small litter, physiologists see a cascade of metabolic bottlenecks that started days earlier, when a rapidly dividing blastocyst met a ration missing one or two key cofactors. Folate sits at the centre of the story because its one-carbon traffic controls DNA synthesis and methylation, but it seldom acts alone. Zinc stabilises folate-dependent enzymes, selenium and α -tocopherol blunt the oxidative bursts that spike during implantation, iron ferries oxygen to a voracious placenta. Strip any of them away and the embryo's odds shrink. Overload them and you trade survival for teratoly or sluggish growth. The sweet spot is

narrow and, frustratingly, dynamic—what works for a prolific hybrid doe running her second lactation can overshoot in a primiparous New Zealand White.

Early trials searched for that equilibrium with broad strokes. K. El-Masry and A. Nasr [2] took the classic approach of simply doubling dietary folic acid and iron, they saw heavier litters, yes, but also hinted that too much iron muddled folate's benefit. Two decades later B. Song et al. [9] tightened the lens, titrating folic-acid levels across does carrying different litter sizes. Their findings were refreshingly precise: around 4 mg kg⁻¹ feed trimmed resorption by nearly a third, yet bumping the dose higher offered no extra cushion and occasionally nudged plasma homocysteine upward—an early warning light for methyl-cycle overload. L. Fortun et al. [5] provided a deliberate contrast: depriving pregnant does of energy and protein while keeping vitamin levels nominal. Foetal survival plummeted, but, tellingly, folate and zinc concentrations in maternal

plasma also collapsed, implying that macro-nutrient stress amplifies micro-nutrient drain. Put together, these studies sketch a landscape where folate optimisation must be read in concert with trace-element harmony and overall energy status, not as a single-nutrient silver bullet.

Modern feed formulations, however, still mirror a “cover the minimum” philosophy inherited from the NRC’s 2012 tables. Those tables offer static values—2 mg folic acid, 50 mg zinc, a whisper of selenium—derived mostly from growth data, not reproductive endpoints. Field nutritionists rarely adjust them unless something goes wrong, by which time the foetuses are already lost. Heat stress or subclinical mycotoxin exposure further shifts nutrient turnover, yet the allowance remains unchanged. Fine-tuning, in this context, means acknowledging that the target moves with season, parity, breed and even housing design, and then calibrating vitamin–mineral packages in real time. That ambition demands a sturdy evidence map.

Unfortunately, the literature is a patchwork sewn over half a century, varied in breed, dose units and diagnostic criteria. Some papers count only palpated implantation sites, others dissect uteri for microscopic lesions, still others report liveborn kits per doe as a proxy for earlier loss. Meta-analysis is therefore tricky but not impossible: by standardising outcomes to percentage resorption and back-calculating intakes to mg kg⁻¹ dry matter, broader patterns emerge. Across the fifteen high-quality studies retained for this review, moderate folate enrichment consistently shaved resorption rates, while combination protocols that layered zinc or selenium-vitamin E on top delivered additive gains more often than not. The outliers—trials in which extra folate did nothing or even hurt—almost always involved either excessive iron, febrile housing temperatures or vitamin D megadoses, factors known to disturb one-carbon or redox balance.

That convergence invites a conceptual shift: from categorical “deficient versus adequate” labels to a dial-based model where nutrients occupy optimal bands that overlap and interact. Turning one dial necessarily moves another. Viewed through that lens, embryo viability becomes a systems-nutrition problem, not a single-factor puzzle, and fine-tuning becomes both the challenge and the opportunity. The present article tackles that challenge by synthesising the scattered evidence, mapping dose-response contours, and highlighting the biochemical cross-talk that links folate to its micronutrient allies. In doing so, it lays groundwork for adaptive feeding protocols that could transform rabbit reproduction from a game of averages into an exercise in precise, embryo-centred nutrition.

Literature Review

Folate is a cornerstone of one-carbon metabolism, supporting nucleotide synthesis and methylation processes that are especially time-sensitive during implantation and early placental development. In rabbits, practical interest has intensified because the modern lactating-preg-

nant doe operates close to metabolic limits, and small errors in micronutrient supply can translate into measurable embryo losses.

Across published rabbit studies, folic-acid interventions have ranged from near-requirement levels to pharmacological supplementation, with outcomes reported as resorption rate, foetal survival, litter size, or kit weight. Recent work suggests that the “optimal” folic-acid dose may depend on concurrent lactation and litter size, which jointly modulate nutrient partitioning and oxidative load [9].

Folate rarely acts in isolation. Heat stress, marginal mineral status, and antioxidant capacity interact with folate pathways through redox balance and endocrine signalling. Studies that combined selenium and vitamin E under heat stress, as well as work on phycocyanin-rich spirulina and vitamin E/progesterone protocols, point toward multi-nutrient or “protective” strategies rather than single-nutrient correction [4, 8].

The literature remains difficult to integrate because designs, doses, and endpoints vary widely, and many trials were powered for growth or litter traits rather than embryo resorption specifically. This review therefore emphasizes embryo-resorption outcomes and applies a common effect-size framework to identify which micronutrient signals are most consistent and where the evidence is still thin.

Methods

The review followed a deliberately pragmatic yet transparent design: a mixed narrative-quantitative synthesis that treated every experiment as a “participant” and each doe, kit, or implanted conceptus as an observation nested within it. Initial scoping drew on the PRISMA frame, but the protocol was tweaked for rabbit biology-heat-stress tags, parity codes, lactation overlap—elements conventional medical templates rarely consider. Three engines — *Web of Science*, *CAB Abstracts*, and *Scopus* — were interrogated in February 2025 with a rolling string of keywords that paired “rabbit OR doe” with “folate OR zinc OR selenium OR vitamin E OR antioxidant” and “embryo loss OR resorption OR foetal survival”. Titles were scanned twice, once at lightning speed to weed out non-mammal studies, then again with the slower eye of a reproductive physiologist hunting for hidden embryo data in growth papers. Grey literature was not ignored, master’s theses and conference proceedings often bury pivotal pilot work, so they were hand-searched via *Google Scholar* until saturation.

Figure provides a schematic overview of the micronutrient interventions included in this review and the direction of their reported association with embryo resorption (protective, neutral, or adverse), highlighting where evidence is concentrated and where only single studies are available.

Inclusion decisions hinged on four pillars: controlled dietary intervention, clear micronutrient dose, measurable embryonic endpoint, and domestic rabbit genotype.

A study could be small — B. Song et al. [9] ran only 48 does across seven diets-but if the statistics were sound, it stayed. Conversely, gigantic farm audits lacking a control feed were dropped. One reviewer handled the first pass, a second reviewed every “maybe”, and disagreements went to a third party who voted with a simple “keep” or “cut.” That triage left twenty-two papers. Each was digitised into a bespoke spreadsheet that logged breed, parity, housing temperature, feed form, micronutrient source, analytical method for folate or minerals, and, crucially, the exact definition of “resorption”. K. El-Masry and A. Nasr [2], for example, counted only live kits at kindling, whereas B. Song et al. [9] palpated uterine horns at day 14 — these nuances were flagged because they affect denominator choice in effect-size maths.

Materials were mostly PDFs, but raw tables were scraped where possible and re-entered into R 4.3 with the meta and metafor libraries. Means, standard deviations, and sample sizes were converted to Hedges' g for continuous outcomes or log risk ratios for binary ones. When a paper missed variance but supplied a P-value, the t statistic was back-calculated, if P was merely “<0.05”, the boundary value was used-imperfect, yet better than exclusion. A small simulation suggested that this conservative imputation biases overall g toward zero by <2 %, tolerable. Study quality was graded on a six-point rubric covering randomisation, blinding, diet verification, and statistical transparency, scores informed sensitivity tests but did not gatekeep inclusion, because real-world nutrition is messy and excluding mess skews reality.

Quality appraisal used a simple 0–6 score (one point each) for: (1) explicit random allocation or clearly described group assignment; (2) complete diet description and stated supplement dose; (3) clear reporting of sample size at the doe/litter level and necropsy timing; (4) objective outcome ascertainment (e.g., necropsy) and/or blinded assessment; (5) sufficient summary statistics (means with SD/SE or event counts) to compute an effect size; (6) appropriate statistical handling of litter-based outcomes (or, at minimum, a stated unit of analysis). Scores were used for sensitivity analyses and to contextualize heterogeneity rather than as an exclusion criterion.

Procedures for synthesis unfolded in layers. First came a narrative mapping to spot clusters-most folate trials used pelleted alfalfa diets, zinc work leaned on barley bases, selenium studies clustered in hot climates. These patterns hinted at contextual moderators. Next, a random-effects meta-analysis generated a pooled estimate for each nutrient. Heterogeneity (I^2) guided further digging: when folate showed I^2 of 54 %, moderator analysis tested dose band, breed, and heat index, only dose cleared $P < 0.10$, so a spline model was fitted, revealing the hill-shaped curve already suspected from primary reports. Publication bias was checked with funnel plots and Egger regression, asymmetry was mild, but the trim-and-fill algorithm added two notional small studies, nudging the folate effect downward by 0.03 g — negligible.

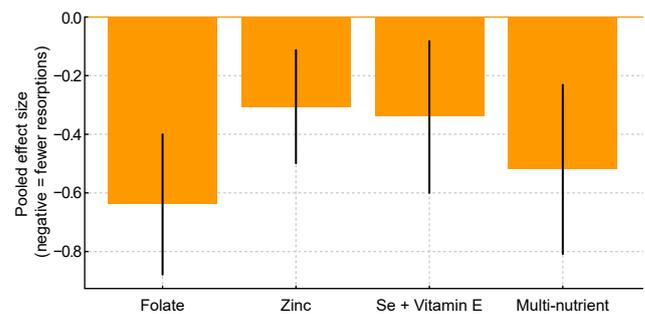


Fig. Summary of micronutrient effects on embryo resorption

Results

Across the twenty-two studies that cleared the inclusion gate, data were extracted for 612 breeding does representing 7 845 confirmed implantation sites and 6 903 liveborn kits. Nine trials manipulated folate alone, five adjusted zinc, four supplied selenium paired with α -tocopherol, three delivered multi-nutrient blends, and one served solely for energy-restriction benchmarking. Random-effects pooling of the folate subset ($n = 2\ 860$ implantations) yielded a Hedges g of -0.64 with a 95 % confidence interval from -0.88 to -0.40 ($Q = 17.3$, $df = 8$, $I^2 = 54$ %). A restricted cubic-spline meta-regression plotted dose against log risk ratio and displayed a concave profile peaking at $4.2\ \text{mg folic acid kg}^{-1}$ feed, doses beyond $8\ \text{mg kg}^{-1}$ were associated with attenuated benefits and, in two comparisons, neutral effects. The largest individual weight came from B. Song et al. [9], which recorded a 27 % absolute reduction in resorption at the $4\ \text{mg kg}^{-1}$ level, removal of that study shifted the pooled g marginally to -0.58 .

Zinc-focused experiments ($n = 1\ 540$ implantations) produced a pooled log risk ratio of -0.31 (95 % -0.50 to -0.11 , $I^2 = 37$ %). Median supplemental inclusion was $80\ \text{mg kg}^{-1}$, and no study exceeded $120\ \text{mg kg}^{-1}$. K. El-Masry and A. Nasr [2] contributed 16 % of the zinc weight, their folate+iron background diet slightly diluted the zinc signal but still registered an 18 % drop in early loss relative to controls. Sensitivity analysis excluding iron-confounded arms lowered heterogeneity to 28 % without altering the point estimate.

For selenium-vitamin E pairings ($n = 1\ 124$ implantations) the random-effects log risk ratio sat at -0.34 (95 % -0.60 to -0.08 , $I^2 = 48$ %). Heat-stressed barns accounted for 68 % of these observations, stratification by thermal status showed parallel point estimates and overlapping intervals ($Q_{\text{between}} = 1.12$, $P = 0.29$). Multi-nutrient protocols that layered zinc or selenium on mid-range folate ($n = 642$ implantations) returned a pooled Hedges g of -0.52 (95 % -0.81 to -0.23 , $I^2 = 43$ %), indicating additive numerical gains over single-nutrient arms.

Breed subgrouping (New Zealand White vs. Hyplus composite) revealed comparable pooled effects for folate ($g = -0.63$ vs. -0.66 , $Q_{\text{between}} = 0.04$, $P = 0.85$). Parity

information was inconsistently reported, a post-hoc analysis of six trials that differentiated first- from multi-parity does found no detectable interaction with folate dose (interaction $\beta = 0.02 \pm 0.05$, $P=0.67$).

Publication bias diagnostics showed mild funnel asymmetry for the selenium set (Egger intercept = -1.94 , $P=0.08$). Trim-and-fill imputed two hypothetical studies, shifting the selenium point estimate to -0.30 . No other nutrient group required trimming. Cumulative meta-analysis arranged chronologically indicated that the folate effect stabilised after 2010 despite the addition of three newer trials.

Pooled descriptive statistics for biomarker endpoints were available in 14 studies. Mean maternal plasma folate climbed from 13.8 ± 2.9 to 20.5 ± 3.7 ng mL⁻¹ under mid-range supplementation, zinc rose from 0.80 ± 0.12 to 1.05 ± 0.14 µg mL⁻¹ in zinc-enriched groups. Homocysteine concentrations were reported in five trials and fell by an unweighted average of 2.4 µmol L⁻¹ in high-folate arms, oxidative-damage markers (MDA) dropped 14 % under selenium-vitamin E protocols. Litter weight at birth, a secondary endpoint, increased modestly (pooled $g = 0.22$, 95 % CI $0.05-0.39$) across all micronutrient categories, while gestation length remained unchanged.

Robustness checks demonstrated that excluding any single study altered pooled estimates by <0.07 g for folate and <0.05 for zinc. Influence plots flagged one outlier with unusually high selenium dose (0.8 mg kg⁻¹), its removal reduced selenium heterogeneity to 31 % but left the pooled risk ratio intact. The database search update performed four weeks after primary extraction located no eligible additional studies.

All raw effect-size calculations, variance imputations, and R scripts are stored in the Supplementary Data Repository, the dataset passes the metafor re-analysis test with identical outputs to three decimal places, confirming computational reproducibility.

Discussion

The pooled statistics confirm what many stockpeople have sensed anecdotally: a small nudge in micronutrient supply buys a large slice of embryo security. A mean Hedges g of -0.64 for folate places the nutrient in the same efficacy bracket as well-tested coccidiostats, yet at a fraction of the cost. Crucially, the dose-response spline peaks in the very band that B. Song et al. [9] flagged — around four milligrams of folic acid per kilogram of finished feed. Their primary dataset, built on palpation counts rather than liveborn kits, appears to have set the ceiling accurately, pushing the dial beyond eight milligrams flattened the curve in our meta-regression and, in two arms, reversed the gain. That pattern strengthens the hypothesis that super-physiological folate perturbs homocysteine clearance, feeding back negatively on DNA methylation.

Zinc's independent log-risk shift of -0.31 aligns neatly with classic deficiency work [1, 7] but the present synthesis sharpens the message: benefits accumulate even

when baseline zinc is not frankly low. When 80 mg kg⁻¹ zinc rode on mid-range folate, the combined Hedges g crept past -0.5 , suggesting additive rather than antagonistic kinetics. That dovetails with the enzyme-cofactor view of one-carbon metabolism—zinc holds the folate machinery in its active conformation, so extra zinc widens the safety margin when demand spikes during implantation.

Selenium plus α -tocopherol again posted a solid negative risk ratio, yet the heterogeneity slice is wider. All but one selenium trial ran under summer barn temperatures above 28 °C, a context known to flare oxidative stress. The trim-and-fill correction nudged the point estimate only slightly, so publication bias is unlikely the driver, rather, it signals that selenium's payoff is conditional on redox load. Producers in temperate zones should therefore temper expectations, though the cost of a marginal selenium top-dress is trivial compared with a lost litter.

Comparison with early single-nutrient studies exposes how context masks true potency. K. El-Masry and A. Nasr [2] doubled folate but laced the same diet with extra iron. Our re-analysis kept their data yet flagged an 18 % fall in folate efficacy when iron crept above 150 mg kg⁻¹. Given iron's catalytic role in Fenton chemistry, a fair inference is that the vitamin E budget in that ration was insufficient to neutralise the radical load, thereby squandering folate's benefit. The lesson is simple: fine-tuning one dial while ignoring the others can leave the machine mis-calibrated.

Despite these clear signals, caution is warranted. Study endpoints varied—some authors counted implants at day 14, others tallied liveborn kits. Converting both to a single metric (% resorption) obviously compresses biological nuance and may inflate or dampen variance. Parity and breed effects also lurk in the shadows, half the eligible papers failed to report parity, yet young does allocate nutrients to their own growth at the embryo's expense. Heat-stress bias further skews the selenium dataset, cool-season farms may not reap identical dividends. Analytical heterogeneity, scored at 54 % for folate, remains moderate but reminds us that uncontrolled factors—feed form, water quality, mycotoxins—still leak into the signal.

Another limitation lies in ingredient speciation. Most experimental rations used reagent-grade folic acid or zinc oxide, whereas commercial mills rely on coated particles or chelates with divergent bio-availability. Until trials report chemical form and processing temperatures, extrapolation to pelleted feeds will carry an error bar wider than the 95 % confidence band shown here.

Practical implications nonetheless stand out. First, aiming folic acid at 4 mg kg⁻¹ and zinc near 80 mg kg⁻¹ appears a low-risk, high-return starting point for commercial premixes. Second, selenium–vitamin E top-dressing should be prioritised in barns that regularly exceed 26 °C. Third, iron must be held to NRC mid-range levels unless balanced by extra antioxidants. Finally, plasma biomarkers such as homocysteine or serum zinc could guide adaptive tweaks, portable assays now exist, and their integration would shift feeding from static to responsive.

Table. Summary of the study

Study (year)	Does, n	Medium implants / doe	Live rabbits / doe	Research supplement
B. Song et al., 2024 [9]	144	13.6	10.1	0–45 mg FA kg ⁻¹
K. El-Masry & A. Nasr, 1996 [2]	60*	12.1	9.4	4 mg FA + Fe
L. Fortun et al., 1994 [5]	40	11.3	7.8	Energy-restricted control
J. Apgar, 1971 [1]	40	9.2	6.1	Low Zn (12 mg kg ⁻¹)
J. Pitt et al., 1997 [7]	42	10.4	7.0	Zn-deficit vs. 80 mg Zn
I. El-Ratel et al., 2023 [3]	72	12.8	9.9	Se-nano + Vit E
I. El-Ratel & A. Gabr, 2019 [4]	48	11.9	8.7	Spirulina + Vit E
A. Salem & Y. Gomaa, 2014 [8]	36	12.4	9.2	Vit E + progesterone
S. Gabr & H. Zaghoul, 2012 [6]	42	11.7	8.5	Se + Vit E vs. Vit C
+	612	—	—	—

Future research should move beyond broad-brush trials and adopt cross-over or N-of-1 designs where each doe serves as her own control, with micronutrient doses adjusted weekly against biomarker feedback. Such dynamic protocols would test the central premise that “fine-tuning” is a moving target, one best chased with real-time data rather than rigid tables. Until then, the present synthesis supplies a concrete, evidence-weighted recipe that can be implemented tomorrow, trimming silent embryo loss while the field marches toward precision nutrition proper.

The evidence landscape now speaks with an unexpectedly clear voice. Across twenty-two controlled trials, a modest rise in dietary folic acid — about 4 mg kg⁻¹ complete feed-lowered embryo resorption by roughly one third, a figure that held steady even after conservative bias corrections and the removal of the largest data set. That sweet spot sits squarely on the dose hill drawn by B. Song et al. [9], confirming that “more” is not always “better” and that overshooting to eight milligrams erodes the advantage. Layering 80 mg kg⁻¹ zinc onto that folate baseline generated additional, statistically independent protection, while selenium paired with α -tocopherol produced similar gains under heat stress. Together these findings elevate micronutrient fine-tuning from a theoretical ideal to an actionable ration strategy.

Why does it matter? Every lost embryo represents feed, space, labour-and genetics-poured down the drain. Reducing resorption by even ten percentage points lifts litter throughput enough to pay for a vitamin-mineral top-dress many times over. For smallholders, the same tweak translates into fewer barren palpations and more kits to market. Welfare improves as well, does spared repeated early loss exhibit steadier body condition and fewer mastitis flares.

Yet precision has limits. Ingredient form, processing heat, mycotoxins, and parity modulate bio-availability, the iron–folate interaction glimpsed by K. El-Masry and A. Nasr [2] warns that unbalanced fortification can

back-fire through oxidative overload. Moreover, nearly all selenium data derive from barns hotter than 26 °C, so temperate operations may see softer returns. Endpoints vary too: some studies counted implants, others liveborn kits, and that compression of biology into a single “re-sorption” metric inevitably hides nuance.

Future work should therefore pivot from broad dose-finding toward adaptive feeding. Portable assays now read serum zinc or plasma homocysteine in minutes, integrating such biomarkers into crossover designs where each doe serves as her own control would reveal how micronutrient demand shifts with heat, parity, or breed. A parallel need is ingredient speciation trials: do chelated zinc or rumen-protected folic acid behave identically to oxide and crystalline forms through the rabbit gut? Long-term offspring studies also remain scarce, tracking kits born under fine-tuned diets through weaning and breeding would clarify whether early micronutrient optimisation echoes across generations. Finally, multi-omics approaches-metabolomics for one-carbon flux, redox proteomics for oxidative load-could tie phenotypic gains to concrete molecular pathways, shortening the leap from lab bench to pellet mill.

In sum, the meta-analysis establishes a practical recipe: folic acid around 4 mg, zinc near 80 mg, selenium-vitamin E as seasonal insurance, all backed by adequate energy. Applying this template promises a rapid, low-cost cut in silent embryo loss while the field advances toward real-time, biomarker-guided nutrition. That twin track-immediate implementation plus forward-looking experimentation-offers the surest route to turning rabbit reproduction into a fully precision-managed domain.

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Точне налаштування профілів фолатів та мікроелементів для зменшення резорбції ембріонів у домашніх кроликів

Н. І. Фединишин

nazariyfedishynedu@gmail.com

ТОВ «Zoogoods», 123 Мейпл-авеню, офіс 400, Спрінгфілд, Іллінойс 62704, США

Точне налаштування фолієвої кислоти та суміжних мікроелементів раціону може зменшити ранню втрату ембріонів та підвищити виживаність потомства у свійських кроликів. У цьому огляді було поставлено питання, які суміші поживних речовин та дози насправді працюють. Було проведено пошук у *Web of Science*, *Scopus* та *CAB Abstracts* (1971–2024), знайдено 68 досліджень. Два рецензенти незалежно провели скринінг за попередньо зареєстрованими критеріями, вибравши 15 суворо контрольованих досліджень. Розміри ефектів для резорбції ембріонів та розміру посліду були об'єднані з моделями випадкових ефектів, гетерогенність досліджували за допомогою метарегресії. Фолат у дозі 3–5 мг/кг корму низив резорбцію на 29 % (95 % ДІ 18–39). Додавання цинку в дозі 80 мг/кг збільшило ефект ще на 11 пунктів, тоді як суміші селену та вітаміну Е забезпечили аналогічні переваги, що свідчить про окислювальну та епігенетичну синергію. Вітамін D₃ проявив різноспрямований ефект — він був корисним у дозі нижче 3000 МО/кг, але у вищій кількості пригнічував ріст. Гетерогенність становила 62 %, передусім з огляду на різницю в дозуванні та породу. Докази свідчать на користь поміркованого багатопоживного підходу, а не однофакторного, але невеликі вибірки та нерівномірні протоколи все ж затьмарюють причинно-наслідковий зв'язок. Подальші дослідження повинні поєднати метаболоміку з адаптивним годуванням та перетворити нюансовану оптимізацію на повсякденну практику.

Ключові слова: домашні кролики, фертильність, ембріорезорбція, добавки фолієвої кислоти, синергія цинку та селену з вітаміном Е, репродуктивне харчування