Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2026.003

RESEARCH ARTICLE

Biodegradable Polycaprolactone/Polyethylene Glycol/Chitosan Intravaginal Implants for Progesterone Delivery: A Preliminary Study on Physicochemical Properties, Release Kinetics, and Biocompatibility

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ARTICLE HISTORY (25-891)

Received: September 14, 2025
Revised: November 28, 2025
Accepted: December 08, 2025
Published online: January 12, 2026

Key words:
Biodegradable polymer
Estrus synchronization
Intravaginal implants
PCL/PEG/Chitosan
Progesterone delivery
Veterinary biomaterials

ABSTRACT

The need for sustainable intravaginal drug-delivery systems in veterinary reproduction is increasing day by day. However, most intravaginal devices currently available in the market rely on non-biodegradable polymers. The aim of this study was to develop and evaluate biodegradable progesterone implants composed of polycaprolactone (PCL), polyethylene glycol (PEG) and chitosan. Three formulations (10/72/18, 20/64/16 and 30/56/14% PCL/PEG/chitosan) were fabricated via melt molding and characterized for morphology, mechanical strength, degradation in simulated vaginal fluid (SVF), progesterone release, cytocompatibility, and environmental compost biodegradation. Statistical analysis was conducted using ANOVA followed, by Tukey's post-hoc test to identify significant differences among implant formulations. Implant I (10/72/18% PCL/PEG/chitosan) demonstrated the highest porosity (11.9%; $P<0.05$), fastest degradation (23% remaining after 10 days of immersion in SVF; $P<0.05$), and a favourable biphasic progesterone release profile with excellent HeLa cell viability (73% after 24h exposure; $P<0.05$). Implant II (20/64/16% PCL/PEG/chitosan) showed steady progesterone release, whereas Implant III (30/56/14% PCL/PEG/chitosan) exhibited an initial burst, followed by prolonged retention. In compost, degradation ranged from complete fragmentation (Implant I) to minimal change (Implant III) after 40 days of burial in compost ($P<0.05$). Preliminary *in vivo* evaluation in four prepubertal Friesian Holstein cyclic heifers confirmed that Implant I was stable, non-inflammatory, and capable of elevating plasma progesterone levels to physiological levels. In conclusion, PCL/PEG/chitosan-based intravaginal system (10/72/18% PCL/PEG/chitosan) is promising biodegradable platform for controlled veterinary hormone delivery and might have potential applications in estrus synchronization in domestic animals.

To Cite This Article: Yessa EY, Santoso, Herdis, Aisah N, Herdianto N, Sitaresmi PI, Gunanti, Wientarsih I, Noviana D, Saidin S, Purwantara B, Amrozi and Ulum MF, 2026. Biodegradable polycaprolactone/polyethylene glycol/chitosan intravaginal implants for progesterone delivery: a preliminary study on physicochemical properties, release kinetics, and biocompatibility. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2026.003

INTRODUCTION

Efficient estrus detection and breeding management are essential tools for maximizing artificial insemination success and overall reproductive performance in cattle (Santos *et al.*, 2025). Estrus synchronization protocols typically employ intravaginal progesterone devices (CIDR/PRID) or prostaglandin-F2α injections. While use

of prostaglandin depends on the presence of a functional corpus luteum and shows variable ovulation responses (Sedó *et al.*, 2022), CIDR devices provide more consistent conception rates through sustained progesterone release (Epperson *et al.*, 2020). However, conventional CIDR/PRID devices are manufactured from non-biodegradable silicone and require to be incinerated or buried after use. This contributes to environmental pollution, carbon emission, and drug residue disposal concerns, and also increases costs. These limitations emphasize the need for sustainable alternatives in accordance with the principles of reduction, reuse, and recycling (Haque *et al.*, 2024).

Biodegradable polymers represent a promising option for intravaginal drug delivery. Polyethylene glycol (PEG) has been widely applied in biomedical devices (Tiboni *et al.*, 2023), polycaprolactone (PCL) offers controlled release but degrades slowly (Ntrivala *et al.*, 2025), and chitosan possesses favourable mucoadhesive properties for vaginal application (Araujo *et al.*, 2021). Together, these materials enable the design of implants capable of releasing progesterone while minimizing the environmental burden.

The main goal of synchronization devices is to maintain progesterone levels above 2ng/mL for 5–7 days, suppress estrus, and enable precise ovulation control (Gobikrushanth *et al.*, 2023). This study aimed to develop a composite biodegradable intravaginal implant combining PEG, PCL, and chitosan for controlled progesterone release and to assess its biodegradability under biological and composting conditions.

MATERIALS AND METHODS

Implant design and fabrication: This study was conducted at the Institut Pertanian Bogor (IPB) University, Indonesia, with geographic coordinates of approximately 6.5567°S latitude and 106.7259°E longitude. Intravaginal implants were designed as rods (12.0×1.5×1.3cm) and prepared in three formulations of PCL/PEG/chitosan (Table 1). Polyethylene glycol (PEG 4000, Merck, Germany) was melted at 150°C for 10–20min using a hot-plate stirrer (Thermo Scientific SP88857105, Canada). Polycaprolactone (PCL, Sigma-Aldrich 704105, USA) was then incorporated and mixed at 100rpm for 15–30min until the mixture became homogeneous. The mixture was cooled to 100°C before the addition of chitosan (PT. Biotech Surindo, Indonesia) and stirred for 5min. After further cooling to 40°C, 1.38g of progesterone (Sigma-Aldrich, Singapore) was added and mixed uniformly. The final blend was cast into molds (Yessa *et al.*, 2023a; 2023b), cooled, wrapped in aluminium foil, and stored at −2°C to 8°C until use.

Table 1: Formulations of PCL/PEG/chitosan intravaginal implants for controlled progesterone release

Implant Number	PCL (wt, %)	PEG (wt, %)	Chitosan (wt, %)	PEG:Chitosan ratio
Implant I	10	72	18	4:1
Implant II	20	64	16	4:1
Implant III	30	56	14	4:1

Note: PCL=polycaprolactone, PEG=polyethylene glycol, wt=weight.

Implant characterization

Morphology and porosity: The surface morphology of implants was analysed using field-emission scanning electron microscopy (FE-SEM; Thermo Fisher Scientific Apreo 2S, UK), as described earlier (Sheela *et al.*, 2022). Prior to imaging, the samples were sputter-coated with a nanometer-scale gold layer to enhance surface conductivity and prevent charging during SEM imaging process. The gold coating was applied using a sputter coater at a thickness of approximately 10nm. Images were acquired at 5.00kV and 500× magnification. Porosity of implants was quantified using ImageJ software by calculating the pore area relative to the total image area (Hojat *et al.*, 2023).

Mechanical properties: The compressive strength was determined according to ASTM D695 using a universal testing machine (GoTech AI-7000S, China), as described earlier by Chen *et al.* (2021). Cylindrical specimens (1.2cm diameter, 3cm thickness) were tested.

Degradation, hormone release and cytotoxicity properties

In vitro degradation in simulated vaginal fluid: Degradation kinetics were assessed using placebo implants (without progesterone) shaped into hemispheres (2.0cm diameter, 1.0cm thickness, 2.17±0.13g). The implants were immersed in 40mL simulated vaginal fluid (SVF), following the procedure of Owen and Katz (1999) at 37.8°C. Weight loss was monitored every two days during the 10-day immersion period.

Compost biodegradation: To evaluate environmental degradation, placebo implants (2.0cm diameter, 1.0cm thickness, 2.3±0.29g) were buried 10cm deep in organic compost (One Home Farm, Indonesia; pH 7; 30–35°C; 40–50% humidity) in 60×35×12cm boxes. Weight loss was recorded every 10 days over the 40-day burial period (Al-Hosni *et al.*, 2019).

In vitro progesterone release: Implants (1.0g; 1.0cm diameter×0.5cm thickness) were incubated in 5mL of 62.5% ethanol at 37°C (Rathbone *et al.*, 2002). To minimize evaporation, the samples were kept fully immersed. Aliquots (1.0mL) were collected at 4, 8, 12, 24, 36, and 48h, diluted (1:3) with 62.5% ethanol to ensure detectable levels for the ELISA assay, and stored at –20°C. The progesterone concentration was quantified using commercially available ELISA kit (DRG, Germany; Catalogue No. EIA-1561). The analytical sensitivity of the assay was 0.045ng/mL, with intra-assay and inter-assay coefficients of variation ranging from 5.4–7.0% and 4.3–10.0%, respectively. The experiments were performed in triplicate.

Cytotoxicity assay: The biocompatibility of the raw polymers (PCL, PEG, chitosan) and the three implant formulations was evaluated by MMT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay using HeLa cells (ATCC CCL-2), as described earlier (Solano *et al.*, 2013). Each material (0.5g of polymer or implant formulation) was incubated in 5mL of Dulbecco's Modified Eagle Medium (DMEM) for 24 or 168h. The extracts were applied to HeLa cells seeded at 5,000 cells/well in 96-well plates and incubated for 24h. The MTT solution is metabolized by viable cells to form purple

formazan crystals. Subsequently, after DMEM was removed, 10µL of MTT solution (5mg/mL) was added for 4h at 37°C with 5% CO_2. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 100µL acidified isopropanol (50% ethanol and 1% acetic acid), followed by incubation at 37°C for 15min with shaking (orbital shaker). The absorbance of the dissolved formazan was measured at 595nm using a Bio-Rad iMark microplate reader (Japan). Cell viability was expressed relative to untreated controls which consisted of cell culture medium without any sample extracts. All tests were performed in duplicate.

Preliminary *in vivo* evaluation in cattle:

Animals: A preliminary *in vivo* study was conducted using six Friesian Holstein (FH) cattle, comprising 1-year-old prepubertal non-cyclic heifer (n=1), 5-year-old non-cyclic primiparous cow with ovarian hypofunction without corpus luteum (n=1), and 1-year-old prepubertal cyclic heifers (n=4), as shown in Fig. 1, maintained at the Reproductive Rehabilitation Unit, IPB University, Bogor, Indonesia (Latitude: -6.556731°, Longitude: 106.725945°). The animals were fed a standardized diet consisting of forage (10% BW/day) and concentrate (2–4% BW/day) with *ad libitum* access to water. All procedures were approved by the Ethics Committee of the Animal Ethics Commission of IPB University (approval No. 001/KEH/SKE/I/2021).

Implant insertion and biodegradation: Considering the results of *in vitro* cytotoxicity, progesterone release kinetics, and biocompatibility, Implant I (10/72/18% PCL/PEG/chitosan) was selected for *in vivo* performance testing due to its superior performance in sustaining progesterone release and exhibiting better cell viability compared to Implants II and III. The Implant I (10/72/18% PCL/PEG/chitosan; 1.38g progesterone) was inserted intravaginally into the six experimental Friesian Holstein females. In non-cyclic animals (prepubertal heifer and primiparous cow), ovarian ultrasound examination was performed two days before implantation to confirm the absence of active corpus luteum (CL), followed by implant insertion on day 0. In prepubertal cyclic heifers, ovarian ultrasound examination confirmed the presence of CL two days prior; therefore PGF2α (5ml Lutalyse™, Zoetis, US, 25mg dinoprost) was injected intramuscularly to regress the CL and reduce progesterone, followed by afternoon implant insertion. Prior to insertion, the vaginal area was cleaned, and baseline measurements (vaginal pH, temperature, and blood collection) were obtained. The implants were monitored for structural integrity and biodegradation using ultrasonography ((Edan DUS 60, rectal probe 3.5 MHz) during the 10-day implantation period, as described previously (Yessa *et al.*, 2023a).

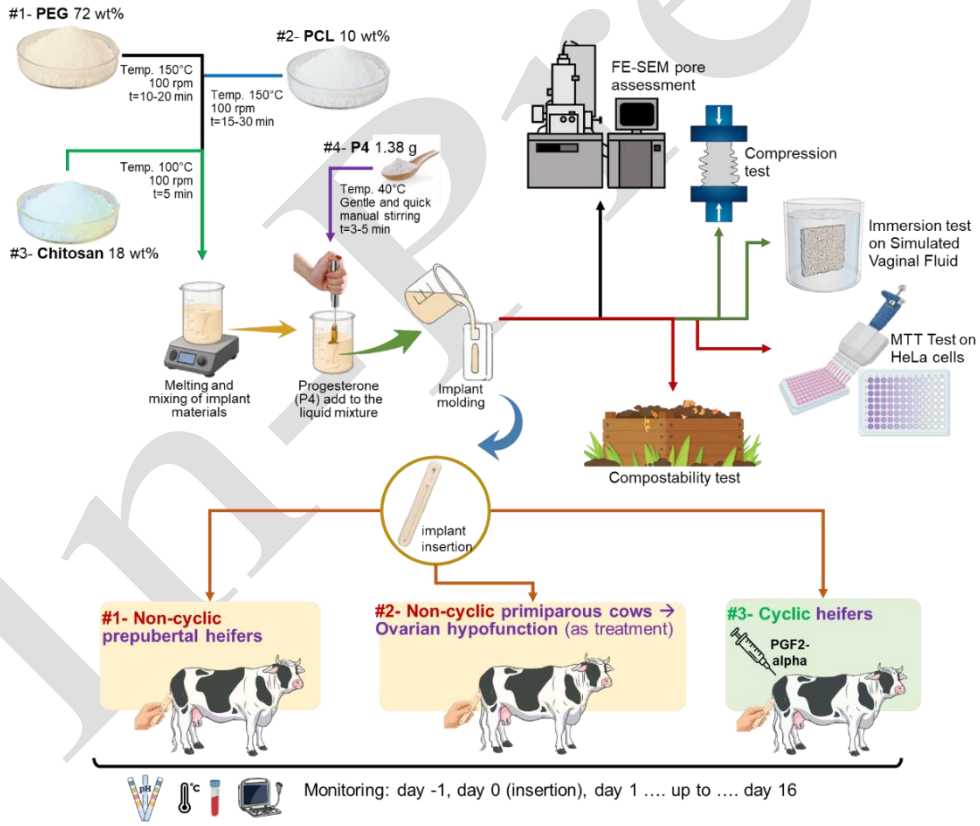

Fig. 1: Schematic diagram of implant fabrication, characterization, *in vitro*, and *in vivo* testing on the non-cyclic prepubertal heifer (n=1), non-cyclic primiparous cow (n=1), and prepubertal cyclic heifers (n=4) of Friesian Holstein breed.

Vaginal pH and temperature: To evaluate potential local effects of implants, vaginal pH was measured using pH indicator strips (Merck, Germany). Vaginal temperature was measured with a digital thermometer (Omron, China) before, during, and after removal of the implant.

Hematology and plasma progesterone: Blood samples (3mL, coccygeal vein) with anticoagulant were collected before and after implant insertion. Samples were divided for hematological analysis and plasma progesterone quantification. Plasma was separated by centrifugation

(3,000rpm, 10min) of blood sample and stored at –20°C until analysis. Progesterone concentrations were determined by ELISA (Yessa *et al.*, 2023a), using commercially available kits (DRG International, Germany, Catalogue No. EIA-1561), as previously described for *in-vitro* progesterone release.

Statistical analysis: This study employed a completely randomized design. Quantitative data were analysed using ANOVA (SPSS 22, IBM, USA), with $P<0.05$ was considered statistically significant, followed by Tukey's post-hoc test for intergroup comparisons. Qualitative data were descriptively analysed.

RESULTS

Three formulations of PCL, PEG and Chitosan intravaginal implants were successfully fabricated in a uniform rod shape (12.0×1.5×1.3cm, 21.70±0.6g). Because they were produced using the same mold, their nearly identical macroscopic appearance made subtle physical differences difficult to distinguish between implants I, II, and III (Fig. 2a–c). The SEM analysis revealed a composition-dependent morphology: Implant I exhibited the highest porosity (11.9%), Implant II had intermediate (3.0%), and Implant III exhibited the lowest (1.1%) porosity ($P<0.05$; Fig. 2d–g). The increased PCL content was associated with denser surfaces and fewer pores. Compressive strength increased proportionally with PCL content, ranging from 2.34 MPa (Implant I) to 4.47 MPa (Implant III), confirming that PCL conferred mechanical reinforcement, the difference was statistically non-significant (Fig. 2h). Spearman's rho analysis showed a significantly negative correlation ($r=-0.683$; $P<0.05$) between compressive strength and porosity, showing higher porosity was associated with lower compressive strength. All implants showed progressive mass loss in the simulated vaginal fluid (Fig. 3a). Implant I degraded most rapidly compared to other implants ($P<0.05$), stabilizing by days 6–8, while Implant III degraded more slowly, consistent with the higher PCL content. In the compost medium, Implant I showed complete fragmentation by day 40, Implant II showed partial fragmentation, and Implant III retained most of its structure with only surface erosion (Fig. 3b). Weight loss was significantly higher for Implants I and II than Implant III on all burial days ($P<0.05$).

In vitro progesterone release kinetics differed among the formulations (Fig. 4a & 4b). Implant I displayed a delayed onset, followed by rapid release; Implant II exhibited steady and controlled release (~60% at 48h), and Implant III showed an initial burst, followed by slower release. The difference in cumulative progesterone release remained non-significant among 3 implants from 24h onward (Fig. 4b).

The MTT assay on HeLa cells indicated higher cytocompatibility of Implant I (>80% viability at 24h, $P<0.05$, and >30% at 168h, $P>0.05$), with lower viability observed for Implants II and III at both time points, though the difference was non-significant at 168h (Fig. 5a & 5b). The PEG exhibited the highest cytotoxicity among the individual components (at 168h, $P<0.05$), while PCL was the most biocompatible at both time points. Morphological analysis confirmed minimal alterations in Implant I–treated cells compared with more pronounced changes in Implants II and III.

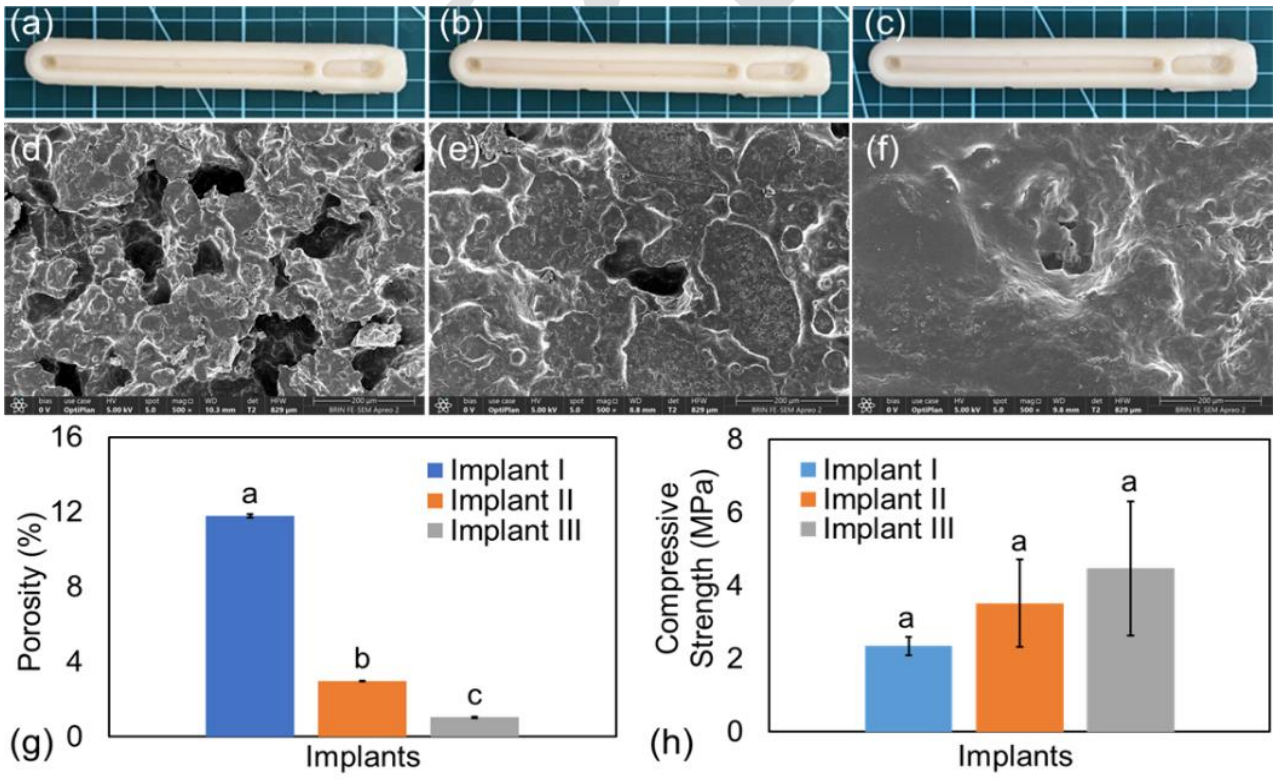

Fig. 2: Vaginal implant of the PCL-PEG-chitosan combination, SEM micrograph and compressive strength characteristics. (a): Implant I (PCL 10%, PEG 72%, chitosan 18%), (b): Implant II (PCL 20%, PEG 64%, chitosan 16%), (c): Implant III (PCL 30%, PEG 56%, chitosan 14%), (d): SEM of implant I, (e): SEM of implant II, (f): SEM of implant III, (g): porosity of PCL-PEG-chitosan implants, and (h): compressive strength of PCL-PEG-chitosan vaginal implant. Bars with different letters indicate significant differences between the groups ($P<0.05$).

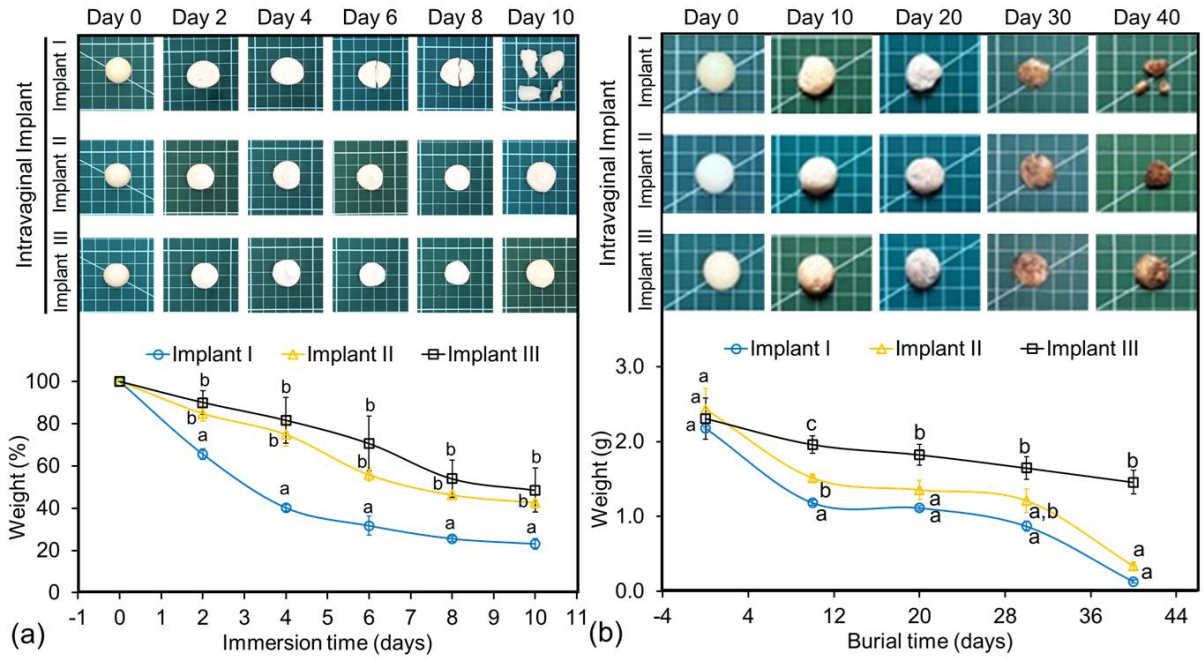

Fig. 3: *In vitro* implant degradation. (a): Implant degradation in simulated vaginal fluid, (b): Implant degradation in compost media. (○) Implant I, (Δ) Implant II, (□) Implant III. Different letters indicate significant differences among the groups (P<0.05).

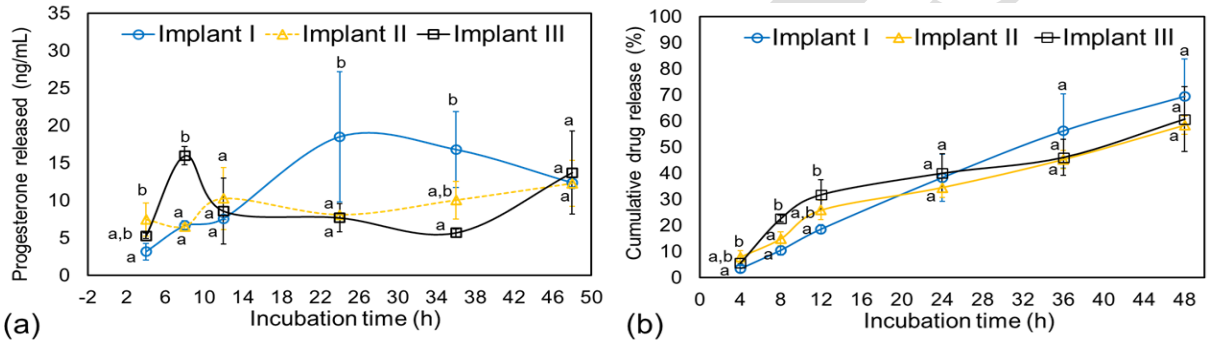

Fig. 4: Properties of progesterone release during immersion test. (a): Actual progesterone release from PCL-PEG-Chitosan implants, (b): Cumulative progesterone release from PCL-PEG-Chitosan implants. (○) Implant I, (Δ) Implant II, (□) Implant III. Different letters indicate significant differences among group values (P<0.05).

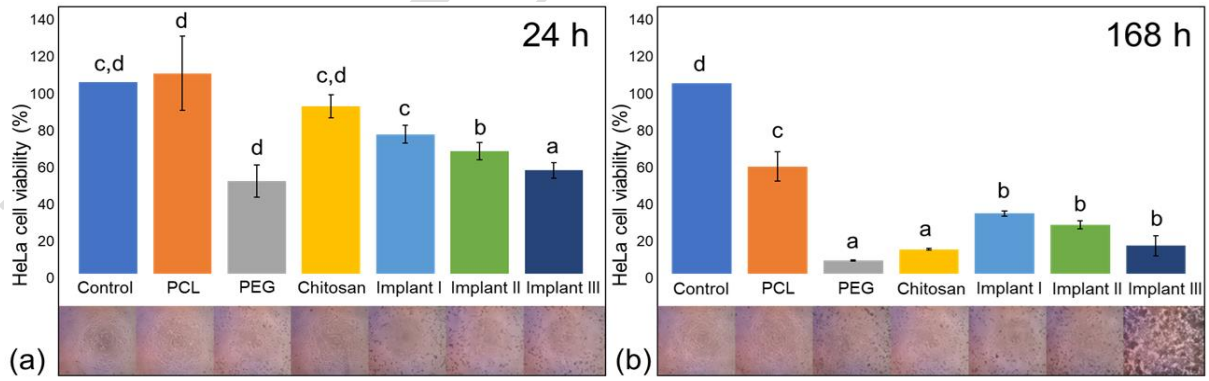

Fig. 5: Viability of HeLa cells after exposure to PCL-PEG-chitosan implant components and Implants; lower panel depicts the morphology of HeLa cells during the MTT assay for materials and implants at 24h (a) and 168h (b). Different letters on bars indicate significant differences among the groups (P<0.05).

Implant I was selected for the *in vivo* testing of non-cyclic prepubertal heifer (n=1), non-cyclic hypofunctional primiparous cow (n=1), and prepubertal cyclic heifers (n=4). The intravaginal implant demonstrated retention for 6–10 days depending on the reproductive status. In non-cyclic prepubertal heifers, structural fracture was visible by day 2 and complete melting occurred by days 8–10 (Fig. 6a-f), while in primiparous cows, melting was completed within 4-5d (Fig. 6g-l). In cyclic heifers, fragmentation occurred within 1–3d, with residual segments adhering to the vaginal mucosa and disappearing by day 5, coinciding with the onset of estrus mucus (Fig. 6m-r).

No significant changes were observed in the width of the vagina, cervix, or uterus before, during, or after Implant insertion (Table 2). Vaginal pH (7.2–7.9) and temperature (37.9–38.9°C) remained within physiological ranges. However, vaginal pH decreased significantly on Day 2 and increased on Day 10 ($P<0.05$) compared to other days of insertion (Fig. 7a). Moreover, vaginal temperature increased ($P<0.05$) before implantation from Day -2 to Day 0, then decreased and remained stable till Day 10 (Fig. 7b).

Haematological analysis indicated that erythrocyte, leukocyte, differential leukocytic counts and other haematological variables did not differ among different time points (Day -1 to 10 of implant insertion) and remained within normal ranges for bovine (Table 3). A transient but significant increase ($P<0.05$) in monocyte count was noted on day 0 but returned to baseline values thereafter. Overall, no haematological abnormalities were detected.

Plasma progesterone monitoring revealed distinct profiles according to reproductive status (Fig. 8). In non-cyclic animals (prepubertal heifer and primiparous cow), no luteal activity was detected during ultrasound scanning before implant insertion and $PGF_{2\alpha}$ injection was not administered (Fig. 8a). The increase in progesterone levels (1-8 ng/dL) after implant insertion in these animals indicated that exogenous progesterone from the implant was well accepted, with progesterone levels correlating with the implant ability to persist in the vagina. In cyclic heifers (Fig. 8b), corpus luteum dynamics and plasma progesterone levels followed the expected physiological patterns, with no significant deviation from controls (animals that did not receive implants), which served as a baseline for comparison. Although progesterone levels decreased after $PGF_{2\alpha}$ injection, they remained above 2ng/mL compared to controls, where levels immediately dropped to basal levels.

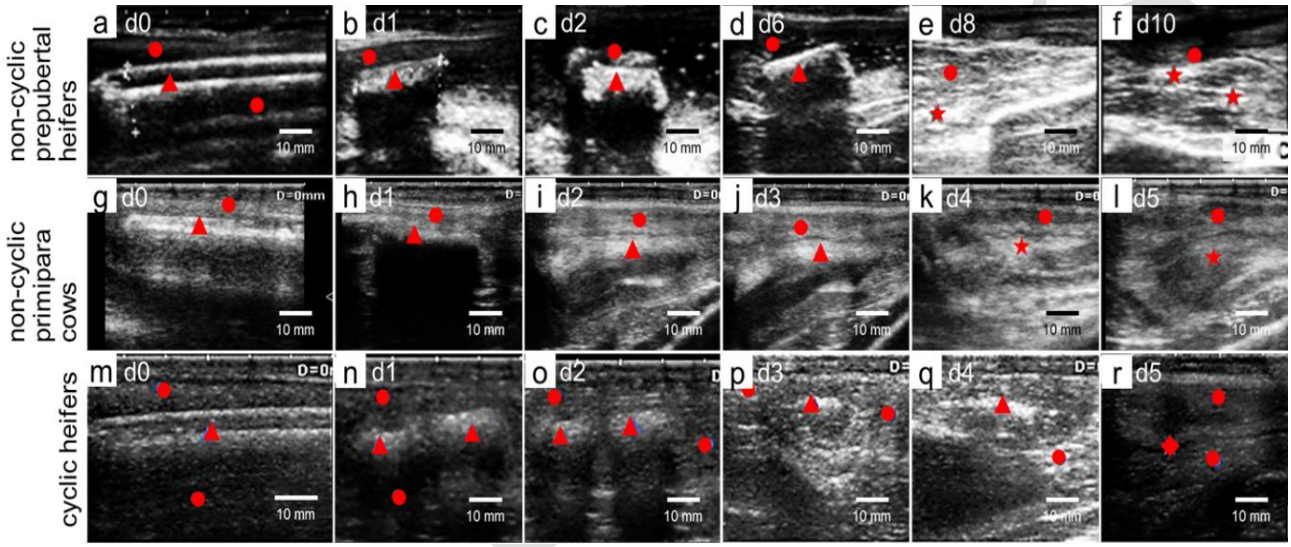

Fig. 6: Temporal morphological changes of Implant I in the vaginal cavity of cattle. (a–f): Sonograms showing ovarian structures in non-cyclic prepubertal heifer (n=1) on day 0–10. (g–l): Sonograms showing ovarian structures in non-cyclic primipara cow (n=1) on day 0-5. (m-r): Sonograms showing ovarian structures in cyclic heifers (n=4) on day 0–5. Note: implant=triangle, implant melt=star, vaginal tissue=circle, and estrous mucus= diamond.

Table 2: Measurements of the vaginal, cervical, and uterine width (cm) before, during, and after vaginal implant I insertion (PCL 10%).

Organ	Days										P value
	-1	0	1	2	3	4	5	6	7	8	
Vagina	3.21±0.4^{a}	2.36±0.6^{a}	2.57±0.3^{a}	2.86±0.4^{a}	2.68±0.1^{a}	3.00±0.5^{a}	3.25±0.3^{a}	3.11±0.3^{a}	2.86±0.5^{a}	2.61±0.2^{a}	0.097
Cervix	2.79±0.1^{a}	2.79±0.1^{a}	2.79±0.1^{a}	2.79±0.1^{a}	2.79±0.1^{a}	2.79±0.1^{a}	2.79±0.1^{a}	2.79±0.1^{a}	2.79±0.1^{a}	2.79±0.1^{a}	0.226
Uterus	3.14±0.2^{a}	3.25±0.1^{a}	3.36±0.3^{a}	3.21±0.4^{a}	3.11±0.5^{a}	3.36±0.5^{a}	3.32±0.3^{a}	3.36±0.3^{a}	3.46±0.1^{a}	3.07±0.5^{a}	0.403

Note: Values followed by the same letter in the same row indicate non-significant differences ($P>0.05$). Day 0 was the day of the vaginal implant insertion.

Table 3: The haematological profile of cattle examined before, during, and after the vaginal implant insertion of Implant I (PCL 10%) in prepubertal cyclic heifers (n=4).

Blood parameters	Days						P-value	Normal value
	-1	0	1	2	3	10		
Erythrocytes (10^6/µL)	5.6±0.2^{a}	5.6±0.2^{a}	5.6±0.4^{a}	5.6±0.4^{a}	5.8±0.2^{a}	5.6±0.3^{a}	0.264	5.0-10.1
Hb (g/dL)	7.7±0.2^{a}	7.7±0.2^{a}	7.9±0.3^{a}	7.6±0.2^{a}	8.1±0.3^{a}	8.0±0.2^{a}	0.108	9.0-13.9
PCV (%)	23.7±0.8^{a}	23.7±0.8^{a}	23.5±0.9^{a}	23.5±0.8^{a}	23.9±0.8^{a}	23.8±0.6^{a}	0.267	28.0-46.0
MCV (fL)	41.5±0.4^{a}	41.3±0.2^{a}	41.5±0.2^{a}	41.6±0.2^{a}	41.9±0.4^{a}	41.0±0.2^{a}	0.367	38.0-53.0
MCH (pg)	13.6±0.2^{a}	13.5±0.1^{a}	13.7±0.1^{a}	13.5±0.0^{a}	13.5±0.1^{a}	13.7±0.0^{a}	0.655	13.0-19.0
MCHC (g/dL)	32.6±0.3^{a,b}	33.2±0.1^{b}	32.6±0.1^{a,b}	32.4±0.2^{a}	32.6±0.2^{a,b}	32.9±0.3^{a,b}	0.204	30.0-37.0
Leukocytes (10^3/µL)	7.7±0.4^{a}	7.7±0.4^{a}	7.7±0.5^{a}	7.6±0.4^{a}	8.8±1.2^{a}	7.0±0.3^{a}	0.963	5.0-16.0
Lymphocytes (10^3/µL)	7.7±1.9^{a}	7.4±0.2^{a}	6.9±0.1^{a}	6.5±0.3^{a}	7.3±0.2^{a}	5.8±0.9^{a}	0.660	1.5-9.0
Monocytes (10^3/µL)	1.1±0.4^{a}	3.9±1.8^{b}	0.8±0.1^{a}	0.8±0.0^{a}	0.8±0.1^{a}	0.8±0.2^{a}	0.050	0.3-1.6
Granulocytes (10^3/µL)	5.3±1.1^{a}	4.8±0.2^{a}	5.1±0.8^{a}	5.3±0.5^{a}	5.4±0.3^{a}	6.2±1.7^{a}	0.942	2.3-9.1

Note: Hb=hemoglobin, PCV=packed cell volume, MCV=mean corpuscular volume, MCH=mean corpuscular hemoglobin, MCHC=mean corpuscular hemoglobin concentration. Values followed by the same letter in the same row indicate non-significant differences ($P>0.05$). Day 0 is the day of vaginal implant insertion.

These findings indicate that the implant was well tolerated intravaginally, underwent complete degradation without inducing pathological changes, and did not disrupt reproductive tract morphology, vaginal microenvironment, or haematological stability.

Cost effectiveness: Table 4 compares three progesterone-releasing implants: the commercial products CIDR and PRID, both non-degradable and requiring removal, and the degradable PCL-PEG-chitosan implant from this study, which dissolved in the vaginal lumen. CIDR costs approximately $21.94 per piece, PRID is priced at approximately $172.42 per piece, whereas the PCL-PEG-chitosan implant costs only $15.29 per piece.

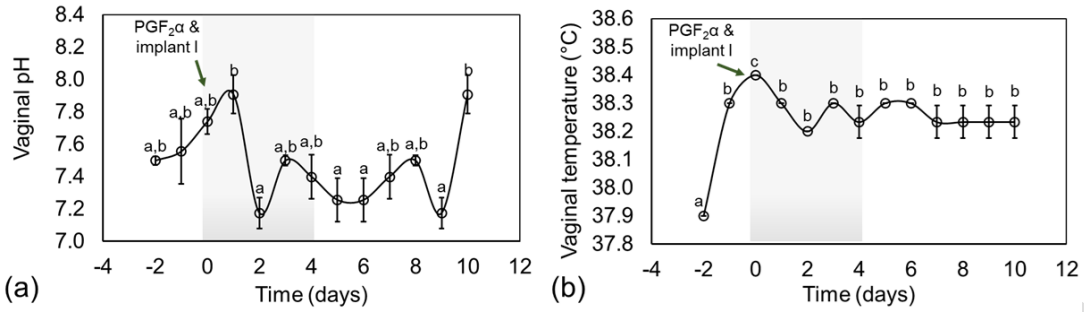

Fig. 7: Vaginal pH levels (a) and temperature (b) measured prior to (2 days), during (4 day), and following the insertion (6 days) of intravaginal Implant I in cyclic heifers. Day 0 marks the point of vaginal implant insertion. Different letters indicate significant differences among the values on different days (P<0.05).

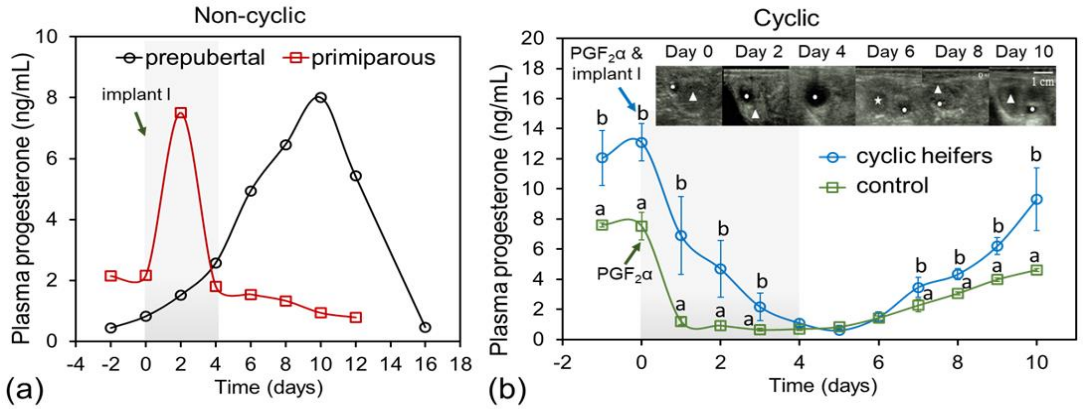

Fig. 8: The concentration of progesterone in the blood plasma of (a): non-cyclic prepubertal heifer (n=1) and non-cyclic primiparous cow (n=1) and (b): prepubertal cyclic heifers (n=4) assessed before and during the 10th-16th day after vaginal implant insertion with Implant I. Note (a): ( ) group of non-cyclic prepubertal heifers (n=1); ( ) group of non-cyclic primiparous cows (n=1). $PGF_2\alpha$ injection and vaginal implant insertion were performed on day 0. Note (b): ( ) group of cows administered PCL-PEG-chitosan implants; ( ) control group without implants (Modification from Sedó *et al*., 2022; n=4). Inset: Ovarian ultrasound images on days 2, 4, 6, 8, and 10 after vaginal implant insertion. Circle=follicle; triangle=CL; star=hemorrhagic corpus. Different letters indicate significant differences among the groups (P<0.05).

Table 4: Physicochemical and functional comparison between commercial intravaginal devices and the present biodegradable implant

Parameters	CIDR (Controlled Internal Drug Release)	PRID (Progesterone-Releasing Intra-vaginal Device)	PCL-PEG-chitosan implant
			
Sources	https://hyperdrug.co.uk/cidr-1-38g-vaginal-delivery-system-for-cattle-pack-of-10/	https://hyperdrug.co.uk/prid-delta-with-grip-tail/	n.a.
Commercial name	EAZY-BREED® CIDR®	PRID® DELTA	n.a.
Company	Pfizer International, USA	Ceva Animal Health, France	n.a.
Packaged (pcs)	10	10	1
Price/pack	£166.79 ~ $219.41	£1,310.65 ~ $1,724.16	IDR 254.550 ~
Per piece	$21.94/pcs	$172.42/pcs	$15.29/pcs
Materials	Porous silicone	Porous silicone	Polycaprolactone/ Polyethylene Glycol/Chitosan
Hormone	Progesterone	Progesterone	Progesterone
Characteristic	Non-degradable	Non-degradable	Degradable
Application	Needs to be removed from vaginal lumen	Needs to be removed from vaginal lumen	Dissolves in the vaginal lumen

DISCUSSION

Implant characterization: The surface morphology of Implant I exhibited a rough and porous structure with a relatively large pore distribution. This porosity increases the available surface area for interaction with cells and body fluids, supporting cell adhesion and migration, as reported for chitosan-based biomaterials (Chang *et al.*, 2023). The predominance of PEG in Implant I further enhances its solubility and flexibility, yielding an open structure (Ghaee *et al.*, 2019). In contrast, Implant II demonstrated a denser surface, attributable to the increased PCL content, which is known to provide rigidity and mechanical stability (Baker *et al.*, 2016; Koch *et al.*, 2022). Implant III, with the highest PCL proportion, appeared smooth and compact, indicating superior structural stability but reduced porosity, which may limit tissue ingrowth. These findings confirm the trend that increasing PCL reduces porosity, while higher PEG increases the openness of the matrix (Ghaee *et al.*, 2019).

Mechanical properties: Compressive strength testing revealed that a higher PCL content improved the mechanical stability, however, the difference among three Implants was non-significant. Implant I (10% PCL) exhibited the lowest strength owing to the dominance of PEG, a hydrophilic polymer that increases flexibility but reduces rigidity (Lai and Chung, 2020). Implant II (20% PCL) showed intermediate strength, whereas Implant III (30% PCL) demonstrated the highest compressive resistance, aligning with the structural role of PCL as a semi-crystalline, load-bearing polymer (Baker *et al.*, 2016; Sani *et al.*, 2021). These results highlight the balance between flexibility (PEG and chitosan) and strength (PCL) in determining implant performance.

***In vitro* degradation and progesterone release:** The degradation rate in simulated vaginal fluid, followed the order of Implant I > Implant II > Implant III. Faster degradation of Implant I was attributed to higher PEG and chitosan contents, both of which are hydrophilic and biodegradable, whereas PCL degrades more slowly (Ramaraju *et al.*, 2025). Implant III degraded more slowly owing to its compact morphology and high PCL fraction.

The progesterone release profiles were also affected by the polymer composition. Implant I demonstrated a faster release owing to its porous structure and high PEG content, which facilitated hydration and pore formation (Lai and Chung, 2020). Chitosan acts as a diffusion regulator, reducing burst release (Chang *et al.*, 2023). Implant II displayed a more stable release pattern, whereas Implant III showed an undesirable burst release, likely caused by non-homogeneous hormone distribution during fabrication.

Cytotoxicity: Cell viability tests indicated that PEG reduced cell viability at 24 and 168h at higher levels compared to PCL and chitosan, which is consistent with previous reports of PEG cytotoxicity (Liu *et al.*, 2017). PCL exhibited the best biocompatibility, as noted in earlier studies (Solano *et al.*, 2013). Chitosan demonstrated intermediate effects, with some reduction in viability over time (Zoe *et al.*, 2023). When implants were considered, Implant I containing high PEG and chitosan content but low PCL content, supported relatively better cell interaction due to its porous structure, whereas the stiffer Implants II and III exhibited limited cell growth at 24 and 168h.

Biodegradation in compost medium: Biodegradation studies of implants in compost medium showed that Implant I degraded most rapidly, followed by Implants II and III, the difference between Implants I and II was non-significant. This trend reflects the influence of PCL, which is known to degrade slowly compared to PEG and chitosan (Stefaniak and Masek, 2021). Blending PCL with hydrophilic polymers enhanced the degradation rate, explaining the faster breakdown of Implants I and II compared to Implant III.

Biodegradation *in vivo*: The intravaginal environment influenced implant degradation differently among animal groups. Implants in non-cyclic heifers degraded more slowly (~10 days) than those in cyclic heifers or non-cyclic primiparous cows (~4 days). The slower degradation in non-cyclic heifers has been attributed to reduced vaginal moisture due to inactive ovarian function and lower estrogen levels (Deng *et al.*, 2019). Unlike non-absorbable devices, such as silicone or Ethylene Vinyl Acetate (EVA), the PCL–PEG–chitosan implants were gradually absorbed, eliminating the need for removal. Unfortunately, these results are based on findings in very few animals (one non-cyclic heifer, one primiparous cow and three cyclic heifers).

Vaginal pH and temperature: Vaginal pH values in experimental animals ranged from 7.2 to 7.9, consistent with reported normal ranges in cattle (Swartz *et al.*, 2014). These near-neutral values indicate a low abundance of Lactobacillus spp., which differentiates cattle vaginal microbiota from humans. In addition, there was an increase in the vaginal temperature before implantation (day −2 to day 0) and a subsequent decrease (day 0 to day 2). Although these changes were statistically significant, the variation represents a normal physiological process reflecting thermoregulatory adaptation in cows, and the temperatures remained within the normal range of the vaginal temperature. This is in line with previous studies in cattle, which showed the average temperature of 38.0±0.8°C in non-estrus (Kim *et al*., 2023) and 39.7±0.5°C in the estrus condition (Polsky *et al*., 2017).

Haematology and immune response: Erythrocyte, leukocyte, lymphocyte, and granulocyte counts remained within normal ranges during the 10-day implantation period (Table 3). Haemoglobin and haematocrit values were slightly below reference ranges but differed non-significantly among different days of Implant I insertion ($P>0.05$). A transient but non-significant leukocyte increase on day 3 likely reflects a mild inflammatory response to implantation. After a transient but significant ($P<0.05$) increase on Day 0, monocyte values normalized after day 1, whereas granulocytes showed increasing trend, though non-significant, until day 10, indicating early but controlled immune activation. Overall stability of the haematological profiles suggested good implant biocompatibility. These findings are supported by previous

evidence that PEG can reduce inflammatory responses, whereas chitosan exhibits anti-inflammatory and immunomodulatory effects (Javdani *et al.*, 2022; Ainun *et al.*, 2024).

Progesterone release: Plasma progesterone levels increased after implantation across groups (non-cyclic prepubertal heifer and primiparous cow), confirming hormone release from the matrix. In non-cyclic prepubertal heifer, progesterone increased steadily until day 10 and then declined to the minimum level on Day 16, as the implant degraded. In the primiparous cow, after attaining peak level on Day 2, progesterone levels started to decline and reached minimum levels earlier (day 12), reflecting faster implant dissolution. Cyclic heifers with implants exhibited progesterone levels >2ng/mL until day 3, after which levels dropped to 0.61±0.07ng/mL on day 5, coinciding with estrus signs. Progesterone levels rose again after day 6, indicating corpus luteum formation. These results demonstrated that the PCL–PEG–chitosan matrix supported effective intravaginal hormone delivery in cyclic heifers. Chitosan contributes to muco-adhesion, whereas PEG improves mucus penetration, facilitating sustained hormone absorption (Wang *et al.*, 2021).

This preliminary study demonstrated that the PCL–PEG–chitosan intravaginal implant, particularly Implant I, exhibited a favourable progesterone release profile and short-term biocompatibility in cattle, indicating its potential as a novel tool for estrus synchronization in non-cyclic, as well as cyclic heifers. The key advantage of this newly developed implant is its economic value. Compared to CIDR and PRID delivery systems, the implant has a lower production cost at USD 15.29 per piece, versus CIDR (USD 21.94 per piece) and PRID (USD 172.42 per piece) costs. This enables widespread use of this implant in smallholder farming systems in developing countries. The PCL–PEG–chitosan implant is fully biodegradable, unlike the non-degradable silicone of CIDR and PRID, eliminating waste costs and environmental burden. These features make it cost-effective for improving cattle reproductive management. Nevertheless, the small sample size, limited observation period, and absence of long-term reproductive performance assessments restrict the generalizability of these findings to the general population. Future studies involving larger cohorts, extended monitoring durations, and optimized fabrication protocols are required to validate the long-term efficacy, biosafety, and environmental impact of this technology. If confirmed, this approach could represent a significant step forward in biomaterial-based veterinary therapeutics, promoting sustainable and cost-effective reproductive management in livestock.

Conclusions: The PCL-PEG-chitosan intravaginal implants demonstrated favourable physicochemical properties, controlled biodegradation, and good biocompatibility. *In vivo* evaluation confirmed its safe application, with stable haematological profiles, physiological vaginal pH, and sustained progesterone release at effective levels. Degradation rates vary with reproductive status, reflecting the differences in local vaginal conditions. Overall, these implants show strong potential as bioresorbable and effective alternatives to conventional intravaginal hormone delivery devices in cattle.

Acknowledgements: The authors gratefully acknowledge the contributions of numerous individuals and institutions that provided valuable support, guidance, and assistance throughout the course of this research. While it is not possible to mention each by name, their collective efforts in facilitating discussions, offering technical expertise, and providing access to essential resources and facilities were indispensable for the successful completion of this study.

Funding declaration: This research was funded by USAID through the Continuing Higher Education Research Alliance Program - CCR ANBIOCORE (by Bambang Purwantara); Postdoctoral Fellowship at the Research Center for Animal Husbandry, National Research and Innovation Agency (BRIN), Indonesia No: B-6996/II.5/SI.06.01/10/2024 (by Elma Yuliani Yessa); and Directorate General of Research and Development, Indonesian Ministry of Higher Education, Science and Technology No: 006/C3/DT.05.00/PL/2025 and the Indonesian Endowment Fund for Education (LPDP) on behalf of the Indonesian Ministry of Higher Education, Science, and Technology that managed under the EQUITY Program No: 4297/B3/DT.03.08/2025 and 42011/IT3/HK.07.00-4/P/B/2025 (by Mokhamad Fakhrul Ulum). This study was also partially funded by Research Organization for Agriculture and Food (ROAF) of the National Research and Innovation Agency (BRIN), Indonesia No: B-34668/III.11/TK. 01.02/12/2024 (by Pradita Iustitia Sitaresmi).

Availability of data and materials: The data that support the findings of this study are openly available in Repository IPB University at https://repository.ipb.ac.id/handle/123456789/136257.

Authors' contributions: Conceptualization of the idea was made by A, BP, and MFU, and methodology was designed by A, BP, MFU, IW, and EYY. The investigation was conducted by EYY, original draft was prepared by EYY and MFU, while review and editing were done by EYY, MFU, and PIS. Visualization by done by EYY and MFU, while the study was supervised by A, BP, MFU, IW, S, H, NA, NH, PIS, G, DN, and SS.

Ethical approval: All procedures related to husbandry and research were conducted in accordance with animal welfare guidelines, as approved by the Ethics Committee of the Animal Ethics Commission, IPB University (Approval No. 001/KEH/SKE/I/2021).

Competing interests: There is no competing of interest by financial, personal, or other relationships with any other person or organization regarding the material discussed in the manuscript.

Use of artificial intelligence tools: Artificial intelligence tools (i.e. ChatGPT and Paperpal) were exclusively utilized to improve the clarity and quality of language without influencing the scientific content.

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