The effectiveness of prostaglandin nanoparticles in corpus luteum regression

D A Kusumaningrum¹, R S Sianturi¹, F A Pamungkas², E Wina¹

¹ Indonesian Research Institute for Animal Production-Ciawi-Bogor
² Indonesian Center for Animal Production and Development-Bogor

Corresponding author e-mail: da_kusumaningrum@yahoo.com

Abstract. Research examined the formation of prostaglandin nanoparticles and their effect on corpus luteum (CL) regression carried out at IRIAP. The nanoparticles formation was carried out using the ionic gelation method. The nanoparticles have a particle size of 316.80±0.14 nm, polydispersion index of 0.453±0.001, zeta potential of +17.40±0.85 mV with 69.69±8.81% hormone entrapment. The effectiveness of nanoparticle in CL regression was observed (prostaglandin vs prostaglandin nanoparticles) using ultrasound observation, hormone profile and estrus response. Further, the size of the ovulating follicle, the time of ovulation, the size of the CL and the onset of estrus after the administration of the prostaglandins were observed. The observation showed that the intramuscular administration of prostaglandin and prostaglandin nanoparticles did not significantly differ on the onset of estrus, time of ovulation, the ovulating follicle size, size of CL and progesterone concentration. The onset of estrus occurred on 2.50 ± 0.58 and 2.33 ± 0.52 days, the ovulation time after hormone administration was on days 3.50 ± 0.55 and 2.83 ± 0.75 with the ovulation follicle size of 16, 62 ± 0.96 and 17.03 ± 1.13 mm, while the CL measures at H-3 were 13.56 ± 2.28 and 10.45 ± 0.88, the progesterone H-2 concentrations were 0.299 and 0.395, respectively for prostaglandin and prostaglandin nanoparticles. It can be concluded that the formation of nanoparticles did not impair the effectiveness of hormones in CL regression so that it can be used to increase the effectiveness of estrus synchronization.

Keywords: Prostaglandin, nanoparticles, corpus luteum

1. Introduction

Nanoparticles (NPs) are substances/material with one final dimension generally ranging from 1-100 nm (10-9–10-7) which are referred to as particles. This size provides them with different physical and chemical properties from materials normally found in the environment [1] hence some materials are considered toxic. Some researchers agree the size of nanoparticles is not more than 1,000 nm [2,3,4]. Meanwhile, in drug delivery, nanoparticles diameter of 200 nm [5, 6], 200–400 nm [7] are recommended for drug delivery systems.

The application of nanoparticles has the opportunity to increase drug efficiency [8]. Some of the advantages of nanoparticles in drug delivery system are their ability to penetrate the intercellular spaces that can only be penetrated by colloidal particle size. The ability to penetrate the cell wall is higher and increases the affinity due to the increased contact surface area by the same amount [9].

Estrus synchronization is a reproductive manipulation technology commonly applied to increase the success of the artificial insemination (AI) program. Application of prostaglandin is usually done to shorten the luteal phase by lysing the corpus luteum [10, 11].
The effects of prostaglandins depend on the phase of the estrus cycle. Prostaglandins given in the middle of the cycle (5-16 days after ovulation) will efficiently induce luteolysis, while if given when no active CL is found (day -3 to +4 from the time of ovulation), estrus synchronization will not work [12]. The study aimed to determine the responses of prostaglandins nanoparticles to CL regression in the estrus synchronization program.

2. Methodology

2.1. Formation of nanoparticle
Prostaglandin nanoparticles were formed using ionic interactions [13] with modifications. Chitosan 0.2% w/v in acetic acid 1% v/v as a source of cations and tripolyphosphate/TPP (0.1% w/v) in sterile water as a source of anions were used in ion exchange reactions. Prostaglandin nanoparticles (0.25 mg/ml) were formed by adding prostaglandins in a chitosan solution and were homogenized using a 1500 rpm magnetic stirrer for 10 minutes. The final pH of the solution formed was adjusted to reach a pH of 5.5 using 1 N NaOH.

2.2. Analysis nanoparticle
The analysis of the particle size and zeta potential was carried out to determine the physical properties of the nanoparticles formed. The mean and distribution of the particle size were measured based on photon correlation spectroscopy (PCS, dynamic light scattering and DLS). Zeta potential was determined based on the electrophoretic light scattering (ELS) technique. The entrapment efficiency (EE) process was determined by centrifugation at 12,000 rpm, and 10°C for 10 minutes to obtain a hormone that was not bound to the nanoparticles (supernatant) and measured using UV spectrophotometry at λ= 260 nm. Entrapment efficiency (EE%) of prostaglandins was calculated as follows.

\[
EE\% = \frac{\text{Total number of prostaglandin - free prostaglandin in supernatant}}{\text{Total Prostaglandin}} \times 100
\]

2.3. In vivo study
This study was conducted to determine the effectiveness of the application of prostaglandin and prostaglandin nanoparticles in regressing the CL.

Treatment 1: Prostaglandin injection (D-11) followed by prostaglandin injection (D0)
Treatment 2: Prostaglandin nanoparticles injection (D-11) followed by prostaglandin nanoparticle (D0)

| Day | PGF2α | PGF2α |
|-----|-------|-------|
| 11  | blood collection 3 day/week | USG - estrous detection |

Figure 1. In vivo study for estrus synchronization in dairy cows using PGF2α protocol two-time injection of PGF2α (11 days intervals).

Monitoring of CL development, follicles, time of estrus (ultrasound observation), concentration of progesterone (blood collection) and onset of estrus was carried out in vivo study. Prostaglandins were injected on day -11 and day 0, and ultrasound (USG) observation was performed every day (start from D0) until an ovulating follicle was found. Blood sampling was carried out every two days until ovulation was found. Ovulation was determined by ultrasound observation characterized by the loss of the dominant follicle. Observation of estrus was carried out after the injection of the 2nd prostaglandins (D-0) to obtain data on onset of estrus. Blood samples were collected to obtain blood plasma for
determination of progesterone concentrations. Blood plasma was collected and frozen until it was ready to be evaluated using the ELISA method. All data in the in vivo study were analysed using a completely randomized design and the differences were tested using LSD.

3. Results and discussion

The physicochemical characteristics of prostaglandin nanoparticles in the form of particle size, polydispersity index (PDI), zeta potential and entrapment efficiency are presented in Table 1.

Table 1. Characteristics of physicochemical properties of prostaglandin nanoparticles.

| Parameters                   | Value       |
|------------------------------|-------------|
| Particle size (nm)           | 316.80±0.14 |
| Polydispersity index         | 0.453±0.001 |
| Zeta Potential (mV)          | +17.00±0.85 |
| Entrapment efficiency (%)    | 69.69±8.81  |

Chitosan in acid media can interact with negatively charged TPP, thereby forming inter- and intra-molecular cross-ionic chitosan nanoparticles [14,15]. The particle size obtained in this study is still in the nanoparticle size range as reported by [4] that nanoparticles are dispersed or solid particles with sizes ranging from 10-1,000 nm, 200-400 nm [7] are recommended for drug delivery systems. Likewise, the polydispersity index value is a measure of the molecular mass distribution in a particular sample. The results showed a PDI value of 0.453 where this value indicates the calculation of the average molecular weight divided by the average molecular weight. The closer to zero, the better the distribution.

Zeta potential is a surface charge that can affect the stability of particles in suspension due to electrostatic repulsion between particles [16]. The results showed that the zeta potential and entrapment efficiency of prostaglandin nanoparticles were +17.00±0.85 mV and 69.69±8.81%. The positive zeta potential (+) a is characteristic of nanoparticles formed from natural polymer chitosan/TPP. In the process of nanoparticle formation, the positively charged amine groups are neutralized by their interaction with the negative charge on the tripolyphosphate molecule and the remaining amino groups result in a positive zeta potential [17]. The higher the zeta potential value in a certain range, the more stable the chitosan nanoparticles formed. The results showed that the prostaglandin nanoparticles formed were quite stable (>20) with fairly good hormone entrapment (69.69±8.81%).

The results of in vivo studies in the form of ovulating follicle size, ovulation time, size of the corpus luteum and onset of estrus after administration of prostaglandin and prostaglandin nanoparticles are presented in Tables 2 and 3 and Figure 2.

The application of prostaglandin nanoparticles did not cause a significant difference in CL size and onset of estrus (Table 2). CL size decreased from day 0 to day 4 after injection of prostaglandin and prostaglandin nanoparticles. CL size decreased from 21.83 ± 2.85 to 7.66 ± 1.45 mm for prostaglandin nanoparticles and 22.80 ± 2.11 to 9.72 ± 0.86 mm for prostaglandin (control). CL regression causes a decrease in progesterone in the blood, resulting in a negative feedback that stimulates gonadotropin release which causes growth and maturation of follicles.

Application of prostaglandin and prostaglandin nanoparticle showed that there was no significant difference between prostaglandin (control) and prostaglandin nanoparticles on the ovulating follicle size and ovulation time (Table 3). Ovulation occurred on day 3.50 ± 0.55 and 2.83 ± 0.75 with an ovulating follicle size of 16.62 ± 0.96 and 17.03 ± 1.13 mm respectively on the application of prostaglandin (control) and prostaglandin nanoparticle. The timing of ovulation is related to the diameter of the dominant follicle. The diameters of the ovulating follicles were 11.1-14.4 mm [18,19] and 10.6-17.6 mm [20].
Table 2. Size of the CL and onset of estrus after intramuscular administration of prostaglandin (control) and prostaglandin nanoparticles.

| Treatment | N | Animal in Estrus | Size of CL (mm) | Onset of Estrus (Days) |
|-----------|---|------------------|----------------|---------------------|
| A         | 6 | 4                |                |                     |
| B         | 6 | 6                |                |                     |

|   | D0     | D1     | D2     | D3     | D4     |
|---|--------|--------|--------|--------|--------|
| A | 22,80±2,11 | 19,56±2,43 | 15,46±1,98 | 13,56±2,28 | 9,72±0,86 | 2,50±0,58 |
| B | 21,83±2,85 | 17,52±1,91 | 14,27±1,91 | 10,45±0,88 | 7,66±1,45 | 2,33±0,52 |

A: Prostaglandin (control)  
B: Prostaglandin nanoparticles  
D-0: Day of the second injection of prostaglandin or prostaglandin nanoparticle  
D1-D4: Day 1-4 after the second injection of prostaglandin or prostaglandin nanoparticle

Table 3. The ovulating follicle size and ovulation time after intramuscular administration of prostaglandin (control) and prostaglandin nanoparticles.

| Treatment | N | Animal in Ovulation | Follicle Size (mm) | Time of ovulation (Day) |
|-----------|---|---------------------|--------------------|------------------------|
| A         | 6 | 6                   | 14,42±1,33         | 3,50±0,55              |
| B         | 6 | 6                   | 13,63±2,66         | 2,83±0,75              |

A: Prostaglandin (control)  
B: Prostaglandin nanoparticle  
D0: Day of the second injection of prostaglandin or prostaglandin nanoparticle  
D1: Day 1 after the second injection of prostaglandin or prostaglandin nanoparticle

Figure 2. Concentration of progesterone (ng/ml) after administration of prostaglandin.

Progesterone concentrations decreased after application of prostaglandin and prostaglandin nanoparticles. There was no significant difference in progesterone concentrations in the two treatments (Figure 2). Corpus luteum plays a role in producing progesterone hormone. Application of prostaglandins causes CL lysis so that the progesterone concentration will decrease to 0.5 ng/ml within 24 hours. CL is responsible for the heat cycle phenomenon [21,11]. The use of prostaglandin hormones...
in estrus synchronization is only effective if livestock have CL or are in the luteal phase. PGF2α will reduce the concentration of progesterone in the blood so that the ratio between the concentration of estrogen and progesterone increases thus cattle will show a pattern of sexual behaviour and ovulation [22].

4. Conclusion and suggestion
The formation of prostaglandin nanoparticles at a particle size of ±300 nm did not change its function and role in regressing CL.

There were no significant changes in parameters (CL size, follicle, onset of estrus, ovulation time and progesterone concentration) that supported the estrus synchronization program thus the formation of prostaglandin nanoparticles at a particle size of 300 nm could be developed to increase the effectiveness of estrous synchronization.

It is necessary to improve the nanoparticle formation method (to increase stability and hormone entrapment) and to test the effectiveness of the application of prostaglandin nanoparticles in large scale of estrus synchronization.

References
[1] Exbrayat JM, Elara N. Moudilou, and Emmanuel Lapied. 2015. Harmful Effects of Nanoparticles on Animals. *Journal of Nanotechnology*. **Volume 2015**, p:1-10. Article ID 861092, 10 pages http://dx.doi.org/10.1155/2015/861092
[2] Tiyaboonchai W. 2003. Chitosan nanoparticles: A promising system for drug delivery. *Naresuan Univ. J.* **11**(3): 51-66.
[3] Buzea C, Blandino IIP, Robbie K. 2007. Nanomaterial and nanoparticles: sources and toxicity. *Biointerphases*. **2**: MR170–MR172
[4] Mohanraj VJ, Chen Y. 2006. Nanoparticles – A Review. *Tropical Journal of Pharmaceutical Research*. **5**(1): 561-573.
[5] Biswas AK, Islam MR et al., 2014. Nanotechnology based approaches in cancer therapeutics. *Adv. Nat. Sci. Nanosci. Nanotechnol.* **5**, 043001.
[6] Rizvi Syed AA, Saleh AM. 2018. Applications of nanoparticle systems in drug delivery technology. *Saudi Pharmaceutical Journal*. **26**: 64-70. doi.org/10.1016/j.jsps.2017.10.012.
[7] Abdassah M. 2017. Nanopartikel dengan gelasi ionic. *Farmaka*. Volume **15** Nomor 1:45-52. https://doi.org/10.24198/jf.v15i1.12138.
[8] Hu M, Li X. 2011. Oral Bio Avaibility: Basic Principles, Advance Concept, and Application. Hoboken (NJ-US): John Wiley & Sons, Inc. p. 32-33.
[9] Martien R, Adhyatmika, Irianto IDK, Farida V, Sari DP. 2012. Perkembangan teknologi nanopartikel sebagai sistem penghantaran obat. *Majalah Farmaseutik*. Vol. **8** No. 1 Tahun 2012.
[10] Macmillan KL, Segwagwe BV, Pino CS. 2003. Associations between the manipulation of patterns of follicular development and fertility in cattle. *Anim. Reprod. Sci.* **78**: 327-344.
[11] Senger, P.L. 2005. Pathways to Pregnancy and Parturition, 2nd edition. Current conception. Inc., Moscow.
[12] De Rensis F, Lo’Pez-Gatius. 2007. Protocols for synchronizing estrus and ovulation in buffalos (Bubalus bubalis): A review. *Theriogenology*. **67**: 209–216.
[13] Wang, X.M, N. Chi, X. Tang. 2008. Preparation of estradiol chitosan nanoparticles from improving nasal absorption and brain targeting. *European J. Pharma.* Biopharma. **70**: 735-740.
[14] De Campos AM, Sanchez A, Alonso MJ. 2001. Chitosan nanoparticles: A new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to cyclosporin A. *International Journal of Pharmaceutics*. **224**(1–2): 159–168.
[15] Xu Y, Du, Y. 2003. Effect of molecular structure of chitosan on protein delivery properties of chitosan nanoparticles. *International Journal of Pharmaceutics*. **250**(1): 215–226.
[16] Qi L, Xu Z, Jiang X, Hu C, Zou X. 2004. Preparation and antibacterial activity of chitosan nanoparticles. *Carbohydrate Research*. **339** (16): 2693–2700.

[17] Urrusuno RF, Romani D, Calvo P. 1999. Development of a freeze-dried formulation of insulin-loaded chitosan nanoparticles intended for nasal administration. *STP Pharm. Sci*. **5**: 429–436.

[18] Safilho MF, Crespilho AM, Santos JEP, Perry GA, Baruselli PS. 2010. Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled Bos indicus cows. *Anim. Reprod. Sci*. **120**: 23–30.

[19] Perry GA, Smith MF, Lucy MC, Green JA, Parks TE, Macneil MD, Roberts AJ, Geary TW. 2005. Relationship between follicle size at insemination and pregnancy success. *Proc. Natl. Acad. Sci. U.S.A.* **102**: 5268–5273.

[20] Cavalieri J, Hepworth G, Macmillan KL. 2004. Ovarian follicular development in Holstein cows following synchronisation of oestrus with oestradiol benzoate and an intravaginal progesterone releasing insert for 5–9 days and duration of the oestrous cycle and concentrations of progesterone following ovulation. *Anim. Reprod. Sci*. **81**: 177–193.

[21] Hafez, E.S.E., B. Hafez. 2001. Reproduction in Farm Animals. 7th edition. Lippincot Williams & Wilkins. Philadelphia

[22] Santos JEP, Thatcher WW, Pool L, Ovtron MW. 2001. Effect of human chorionic gonadotropin on luteal function and reproductive performance of high producing lactating Holstein dairy cow. *J Anim Sci*. **79**: 2881–2894

**Acknowledgments**

The authors would like to thank the all parties who have helped to carry out this research. We appreciate the Agency for Agricultural Research and Development which has funded this research through the National Budget IRIAP-ICARD.