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Research Article

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2025.56.3.444-449

Efficacy of progesterone nano cream (ProSync- NC) on oestrus induction and fertility outcomes in postpartum crossbred dairy cows#

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Citation: Rini, M.M., Benjamin, E.D., Jayakumar, C., Becha, B.B., Abhilash, R.S., Mohan, K.M.S, Unnikrishnan, M.P. and Saifudeen, S.M. 2025. Efficacy of Progesterone nano cream (ProSync- NC) on oestrus induction and fertility outcomes in postpartum crossbred dairy cows. *J. Vet. Anim. Sci.* **56** (3):444-449

Received: 21.02.2025 Accepted: 17.05.2025 Published: 30.09.2025

Abstract

This study aimed to evaluate the efficacy of progesterone nano cream (PNC) to induce oestrus and conception rate in crossbred dairy cows. Twenty four crossbred dairy cows were randomly divided into two groups on day 60 postpartum on the basis of presence or absence of corpus luteum viz. with CL and without CL, each group was further divided into treatment and control, with six animals in each subgroup. On Day 0 (day 60 postpartum) in treatment animals (group1- with CL and group 2- without CL) PNC was applied on depilated skin of forelimb below knee and secured with an adhesive and water resistant bandage. On day 6, Inj. Cloprostenol 500 µg (IM) was administered and on day 7, PNC bandage was removed. Control animals (group 3- with CL and 4- without CL) were observed over a period of 21 days from day 60 postpartum without any treatment. The oestrus induction response of group 1 and 2 was 100 and 83.33 per cent, respectively. The interval from PNC removal to induced oestrus was significantly ($p < 0.01$) lower in group 1 (54.60 ± 8.99 hours) than group 2 (118.80 ± 9.56 hours). Higher percentage of animals in control group displayed intense grade oestrus. The duration of oestrus in group 1 (56.00 ± 7.16 hours) and group 2 (85.80 ± 13.41 hours) was significantly ($p < 0.05$) longer than the control group 3 (30.33 ±1.45 hours) and group 4 (32 hours). The overall conception rate in group 1, 2, 3 and 4 were 83.33, 50, 66.67 and 50 per cent, respectively. The calving to first service interval was significantly ($p < 0.05$) lower in the treatment groups, and the calving to conception interval was also shorter compared to the control groups. It is concluded that PNC could effectively induce oestrus even in cows without ovarian rebound and thereby restoring ovarian cyclicity and shortening the intercalving interval.

Keywords: *Progesterone nano cream, intercalving interval, oestrus induction, ovarian cyclicity*

Efficient reproductive management in dairy cows is crucial for maintaining optimal milk production and economic sustainability in the dairy industry. One of the key factors influencing reproductive efficiency is the intercalving interval (ICI), which directly affects herd productivity. According to Lopes *et al*. (2009), a shorter ICI of around 365 days was associated with higher profitability, as it maximised the number of calvings per year, leading to increased milk production. For achieving an ICI of 365 days animal should ideally conceive within 85 days postpartum. Extended ICI occupies

#Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

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46.1 per cent of the total cost incurred for dealing with reproductive problems (Deka *et al*., 2021). Arti *et al*. (2019) state that an ICI extending to 18 months in a dairy farm would lead to unprofitability. Anilkumar *et al*. (2023), reported an ICI of 551 ± 48 days in crossbred cows in Kerala. Prolonged ICI is often associated with delayed ovarian rebound, postpartum anoestrus, and failure to exhibit oestrus, despite proper nutritional and management practices. Ideally, cows should return to oestrous cyclicity within 60 days postpartum, allowing timely breeding and reducing ICI. However, many crossbred dairy cows experience delayed ovarian rebound, particularly due to metabolic stress, or postpartum complications. Studies have shown that even with proper management, a significant proportion of cows fail to exhibit oestrus, leading to longer calving to conception intervals and economic losses.

Progesterone based therapies have been widely used for oestrus induction and synchronisation, particularly in cows exhibiting postpartum anoestrus or silent heat. Secondary complications of vaginitis and copious vaginal discharge are sequalae to the conventional intra-vaginal inserts (Laven *et al*., 2000). Among these, Progesterone Nano Cream (ProSync-NC) offers a novel, non-invasive transdermal approach for delivering sustained progesterone levels, mimicking the natural luteal phase, and triggering subsequent follicular development.

Materials and methods

Twenty four postpartum crossbred dairy cows maintained under uniform management conditions at the University Livestock Farm and Fodder Research and Development Scheme, Mannuthy, Kerala Veterinary and Animal Sciences University, Kerala, were utilised for the study. The study commenced on day 60 postpartum in animals which were devoid of any pre or postpartum complications. A detailed clinico-gynaecological examination was performed in these cows to rule out any uterine pathology. Per rectal and transrectal ultrasonographic examination (Mylab™ Gamma, EsoateSpA, Italy using B-mode equipped with linear array, 5-10 MHz frequency transrectal transducer) was carried out to assess the ovarian status before initiation of the treatment. The grouping was done based on the presence or absence of the corpus luteum (CL) on either ovaries. Animals with CL on either ovaries were randomly allocated to treatment (group 1) or control (group 3) whereas, animals without CL on either of their ovaries were randomly allocated to treatment (group 2) or control (group 4), with six animals in each group.

Experimental design

In treatment groups 1 and 2, animals were applied with PNC on day 60 postpartum (D0) on the depilated skin in the forelimb below knee. Then covered with an adhesive bandage and secured with a water resistant bandage and was retained for seven days. On day six, 500 µg of $PGF_{2\alpha}$ analogue, Cloprostenol sodium (IM) was administered. After 24 hours the PNC bandage was removed. On the detected oestrus artificial insemination (AI) was performed using frozen thawed semen.

In control groups 3 and 4, no treatments were given and the cows were observed for exhibition of natural oestrus over a period of 21 days starting from day 60 postpartum. AI was performed on natural oestrus with frozen thawed semen.

The characteristics of induced oestrus were observed in treatment groups and compared with that of control. The oestrus induction response in the treatment groups, the interval from the removal of PNC to the onset of oestrus signs was recorded as the time taken for induction of oestrus. The period from the beginning to the end of the exhibition of oestrous signs was recorded as the duration of oestrus. The intensity of the oestrus was assessed based on behavioural changes, physiological changes and gynaecological observations, which was scored as intense, intermediate and weak as described by Azeez (2014). Serum progesterone values were estimated using chemiluminescent immunoassay (CLIA) and compared between groups on day 0, 7 (between treatment groups only), day of oestrus and day 12 post oestrus. Pregnancy was confirmed in all the groups at day 45 post-AI using transrectal ultrasonography. The first service conception rate and overall conception rate was calculated as the percentage of animals conceived with first AI and in three consecutive AIs out of the total number of cows inseminated in each group. Calving to conception interval (CCI) was calculated for all the groups and compared. Descriptive statistics were computed for all variables. The data on oestrus induction and conception rates (CR) were analysed by chi-square test and time taken for onset of oestrus, duration of oestrus, and calving to conception interval were calculated by one-way ANOVA.

Results and discussion

Parity distribution of animals

At day 60 postpartum, 75 per cent of pluriparous cows had a CL, while 25 per cent did not. In contrast, only 33.33 per cent of primiparous cows had a CL, whereas 66.67 per cent lacked CL. The higher per centage of pluriparous cows with CL and primiparous cows without CL suggests a longer ovarian resumption period in primiparous cows, aligning with the findings of Tanaka *et al*. (2008) and Crowe *et al*. (2014).

Oestrus induction response

In group 1, 100 per cent (6/6) and in group 2, 83.33 per cent (5/6) of cows exhibited oestrus. However, in the control group 3 and 4, only 50 and 16.67 per cent, respectively exhibited oestrus during the 21 days observation period from day 60 postpartum (Table 1).

Statistical analysis of the data revealed a significant association ($p < 0.05$) between the treatment and oestrus induction response, implying the efficacy of PNC in oestrus induction. Similar finding was made by Dhami *et al*. (2015), using CIDR + $PGF_{2\alpha}$ with 100 per cent oestrus induction response. Similarly, Lakshmikantan *et al*. (2021), reported a 100 per cent oestrus response using progesterone coated nano fibre dermal patch (ProSync-NF). Similar observation on the oestrus response was also reported by Van Werven *et al*. (2013), using intravaginal devices for oestrus induction.

Time taken for induction of oestrus

The mean time taken for induction of oestrus from the removal of PNC in treatment group 1 (54.60 ± 8.99 hours) was significantly ($p < 0.01$) shorter compared to group 2 (118.80 ± 9.56 hours) (Table 1). These results were consistent with the findings of Romano and Fahning (2013) and Stevenson (2008), who reported that, the average time taken for the onset of oestrus was 56.3 ± 17.2 and 96 hours, respectively after the removal of progesterone source. The mean time for oestrus induction after PNC removal aligns with the findings of Chebel *et al*. (2006), who reported oestrus onset within two to four days following progesterone insert removal. Similarly, Putro (2013) observed that intravaginal progesterone implants (CIDR) induced oestrus approximately 66 hours post-removal, especially when combined with $PGF_{2\alpha}$. Lakshmikantan *et al*. (2021) reported that using a progesterone-coated nanofiber dermal patch (ProSync-NF) resulted in 71.4 per cent of animals exhibiting oestrus within 3 days, while 28.6 per cent showed oestrus at 5 days after transdermal patch removal. The difference in time taken for oestrus induction in treatment groups might be due to the difference in the stage of follicular wave at the end of treatment (Roche *et al*., 2000).

Duration of oestrus

The mean duration of oestrus in treatment group 1 (56.00 ± 7.16 hours) was significantly ($p < 0.05$) shorter compared to group 2 (85.80 ± 13.41 hours) (Table 2). This extended duration of oestrus might be due to the higher levels of progesterone in the preovulatory period that delayed LH surge and extended oestrus duration (Singh *et al*., 2012). In the present study, higher serum progesterone (ng/ mL) on the day of oestrus in treatment groups 1 (1.03 ± 0.36) and 2 (0.94 + 0.19) compared to group 3 (0.89 ± 0.21) and 4 (0.80) might have caused the extended duration of oestrus as it blocked the LH surge and delayed the ovulation (Niyas, 2017).

Table 2. Duration of oestrus (Mean ± SE) in treatment and control groups

Group (n=6)	No. of animals that exhibited oestrus	Duration of oestrus (hours)
G 1	6	$56^{ab} \pm 7.16$
G 2	5	$85.80^{a} \pm 13.41$
G 3	3	$30.33^{b} \pm 1.45$
G 4	1	32.00
F value		6.463
P value		0.014*

* Significant at 5% level

Intensity of oestrus

In the present study, the proportion of animals in group 1 that exhibited intense, intermediate and weak signs of oestrus was 16.67, 66.67 and 16.67 per cent, respectively. In group 2, it was 0, 60 and 40 per cent, respectively. While in control group 3, 66.67, 33.33 and 0 per cent, respectively. In control group 4, 100, 0 and 0 per cent respectively. Thus, in both treatment groups, the per centage of intense, intermediate and weak intensities were 9, 63.63 and 27.27 per cent, respectively, whereas in control groups it was 75, 25 and zero per cent, respectively. This could be due to higher levels of post-treatment progesterone inhibiting oestradiol feedback, thus affecting oestrus intensity (Rajamanickam *et al*., 2024).

Conception rate

The first service conception rates in group 1, 2, 3 and 4 were 16.67, 0, 33.33 and 0, respectively. The reduced first service CR in PNC treated cows (groups 1 and 2) can be attributed to prolonged oestrus, extended follicular persistence, suprabasal progesterone levels, and compromised luteal function, all of which negatively impact fertility outcomes. Similar lower CR in anoestrous cows treated with progesterone due to suboptimal hormonal balance and delayed ovulation have been reported. Pursley

Table 1. Oestrus induction response and time taken for onset of oestrus (Mean ± SE) in treatment and control groups

Group (n=6)	Oestrus induction		Time taken for onset of oestrus	
	No. of cows responded	Percentage (%)	From Day 0 (days)	From Day 7 (hours)
G 1	6	100	$9.20^{b} \pm 0.37$	$54.60^{b} \pm 8.99$
G 2	5	83.3	$11.80^{a} \pm 0.37$	$118.80^{a} \pm 9.56$
χ^2/t value		χ^2=10.489	t = 4.914	t = 4.890
P value		0.015*	0.001**	0.001**

* Significant at 5% level

** Significant at 1% level

and Martins (2012) reported lower fertility in progesterone induced oestrus cows due to prolonged follicle growth and poor oocyte competence. Bisinotto *et al*. (2010) noted reduced conception rates in progesterone treated anoestrous cows due to low progesterone concentrations post AI. Wiltbank *et al*. (2011) emphasised that suboptimal progesterone levels during AI reduce embryo survival and overall conception rates. Although the first service conception rate was lower, particularly in PNC treated cows, the overall CR improved across subsequent cycles.

The overall CR after 3 consecutive AI in the group 1, 2, 3 and 4 was 83.33, 50, 66.67 and 50 per cent, respectively (Table 3). This finding was in agreement with Dhami *et al.* (2015), who reported an overall CR of 80 per cent using CIDR protocol. However, statistical analysis revealed no significant difference ($p \geq 0.05$) in conception rate between treatment and control groups, which could be due to the smaller number of animals in each group. Suprabasal progesterone concentrations during oestrus in prolonged oestrous cows negatively impact LH pulsatility, delaying ovulation and leading to the release of aged, less competent oocytes (Singh *et al*., 2009; Niyas, 2017; Arun, 2019; Patel *et al*., 2020). Additionally, reduced IGF-1 secretion and poor follicular cell sensitivity to LH further contribute to lower first service CR (Bage *et al*., 2003; Omari *et al*., 2020). However, the increase in overall CR following multiple inseminations suggests that the influence of suprabasal progesterone diminishes over time, allowing more synchronised ovulation and better oocyte competence in later cycles. Several studies support the gradual improvement in CR over multiple inseminations despite initial challenges. Kasbe *et al.* (2014) found a higher CR (63.63 per cent) in postpartum anoestrous cows treated with progesterone, requiring an average of two services per conception, highlighting improved fertility over successive cycles. Pacala *et al*. (2010) reported lower CR in early inseminations, but fertility improved with repeated AI. Bisinotto *et al*. (2010) noted that progesterone based synchronisation protocols improve fertility in anoestrous cows over multiple cycles, despite initial challenges with first AI. Wiltbank *et al*. (2011) emphasised that suboptimal progesterone levels initially reduce embryo survival, but subsequent cycles benefit from improved ovulatory patterns.

Calving to first service interval

In the present study calving to first service interval in group 1, 2, 3 and 4 was 71.17 ± 1.62, 78.67 ± 4.02, 93.33 ± 10.44 and 148.00 ± 32.20 days. Compared to control groups, the calving to first service interval was significantly ($p < 0.05$) shorter in treatment groups (Table 4). Numerous studies indicate that progesterone based synchronisation protocols significantly improve postpartum reproductive efficiency. Bisinotto *et al*. (2010) demonstrated that CIDR-based protocols effectively shorten the duration of postpartum anoestrus, thereby decreasing the interval from calving to first service. Stevenson and Pulley (2016) found that the combination of progesterone inserts with GnRH and $PGF_{2\alpha}$ enhances oestrous synchronisation, leading to reduced first service intervals and higher conception rates. Wiltbank *et al*. (2011) highlighted that progesterone treatment facilitates follicular turnover and the resumption of ovulation, enabling cows to return to cyclicity more quickly.

Calving to conception interval

In the present study, the mean calving to conception interval (CCI) of group 1, 2, 3 and 4 was 126.80 ± 27.06, 147.00± 8.54, 152.25 ± 34.91 and 164.00 ± 49.51 days, respectively. Although variations were observed in the CCI among the groups, statistical analysis revealed no significant differences between the treatment and control groups. An average reduction of 22 days could be observed between treatment and control groups (Table 4).

The persistence of the dominant follicle in PNC treated cows could have reduced the first service conception rate due to delayed ovulation and poor oocyte quality. However, a better overall CR was observed over multiple inseminations, likely due to the waning effects of prolonged follicular persistence in subsequent cycles. To further improve reproductive efficiency and reduce CCI, modifying the PNC protocol by incorporating GnRH at the time of PNC removal is necessary. This modification can trigger timely LH release, synchronize ovulation, prevent follicular persistence, and enhance corpus luteum function, ultimately leading to higher first service CR and optimized postpartum fertility outcomes in dairy cattle (Pawar *et al*., 2012).

Table 3. First service conception rate and overall conception rate for three AI in treatment and control groups (n=6)

Group	No. of animals	No. of animals inseminated	No. of animals conceived	Per cent of animals conceived	Overall conception rate(n=6)
G 1	6	6	1	16.66	83.33 (5)
G 2	6	5	0	0	50 (3)
G 3	6	3	1	33.33	66.67 (4)
G 4	6	1	0	0	50 (3)
χ^2 value (P value)					2.019 $(0.568)^{ns}$

Figures in parenthesis indicate the number of animals

ns- Non significant

Table 4. Time interval between calving to first service, calving to conception (days)

Group	Time interval between calving to first service	Time interval between calving to conception	
G1	$71.17^{b} \pm 1.62$	126.80 ± 27.06	134.38 ± 16.82
G2	$78.67^{b} \pm 4.02$	147.00 ± 8.54	
G3	$93.33^{b} \pm 10.44$	152.25 ± 34.91	157.29 ± 26.53
G4	$148.00^{a} \pm 32.20$	164.00 ± 49.51	
F value	4.168	0.245	
P value	0.019^{*}	0.864^{ns}	

* Significant at 5% level

ns- Non significant

Conclusion

This study demonstrated the efficacy of Progesterone Nano Cream (PNC) in postpartum reproductive management by improving calving to first service and calving to conception intervals, as well as overall conception rates. However, challenges such as follicular persistence, suprabasal progesterone during oestrus, and lower first service conception rates suggest the need for protocol refinement. The benefits of PNC include shortened postpartum anoestrus and improved conception rates across multiple cycles, making it a viable field application.

Acknowledgements

The authors are thankful to the Dean, College of Veterinary and Animal Sciences, Mannuthy for providing all the facilities to carry out the work.

Conflicts of interest

The authors declare that they have no conflict of interest.

References

Anilkumar, K., Radhika, G., Divya, P., Bimal, C.B. and Aravindakshan, T.V. 2023. Evaluation of reproductive performance of indigenous and crossbred cattle in Kerala: A field level study. *Indian J. Vet. Anim. Sci. Res*. **45**: 482-489.

Arti, A., Sirohi, S. and Oberoi, P.S. 2019. Effect of changes in milk yield and inter-calving period on profitability of commercial dairy farm. *Indian J. Anim. Sci.* **89:** 1411-1412.

Arun, K. P., Nair, S. P. and Niyas, P. A. 2019. Hormonal and ultrasonographic evaluation of reproductive cycles in postpartum dairy cows. *Vet. World*. **12**: 389–394.

Azeez, C.P.A. 2014. Management of anoestrum in crossbred heifers and cows by hormonal induction of oestrus. *Ph.D thesis*, Kerala Veterinary and Animal Sciences University, Wayanad. 147p.

Bage, R., 2003. Conception rates after AI in Swedish red and white dairy heifers: relationship with progesterone concentrations at AI. *Reprod. Dom Anim.* **38**:199-203.

Bisinotto, R. S., Ribeiro, E. S. and Santos, J. E. P. 2010. Effect of hormonal treatments on reproductive efficiency of lactating dairy cows. *Theriogenology.***74**: 622–632.

Chebel, R.C., Santos, J.E., Cerri, R.L., Rutigliano, H.M. and Bruno, R.G.S. 2006. Reproduction in dairy cows following progesterone insert presynchronization and resynchronization protocols. *J. Dairy Sci.* **89**: 4205-4219.

Crowe, M.A., Diskin, M.G. and Williams, E.J. 2014. Parturition to resumption of ovarian cyclicity: comparative aspects of beef and dairy cows. *Animal*. **8**: 40-53.

Deka, R. P., Magnusson, U., Grace, D., Randolph, T. F., Shome, R. and Lindahl, J. F. 2021. Estimates of the Economic Cost Caused by Five Major Reproductive Problems in Dairy Animals in Assam and Bihar, India. *Animal.* **11**:3116.

Dhami, A.J., Nakrani, B.B., Hadiya, K.K., Patel, J.A. and Shah, R.G. 2015. Comparative efficacy of different estrus synchronization protocols on estrus induction response, fertility and plasma progesterone and biochemical profile in crossbred anestrus cows. *Vet. Wld*. **8**:1310.

Kasbe, R.D., Markandeya, N.M., Sawale, A.G. and Pawar, S.A. 2014. Studies on induction of oestrus in post-partum anoestrus local cows with progesterone primed GnRH and progesterone treatment. *Indian J. Anim. Reprod*. **34**(2).

Lakshmikantan, U., Vadivoo, V.S., Geetha, T. and Mathivanan, R. 2021. Effect of progesterone coated nano fibre dermal patch (ProSync-NF) on cattle diagnosed with Anestrus and silent or unobserved estrus. *Indian J. Anim. Sci.* **91**: 5-8.

Laven, R.A., Biggadike, H.J., Proven, M.J., Halfacre, S. and Tickle, L.R. 2000. Changes in vaginal microbiology associated with the use of progesterone-releasing intravaginal devices. *Vet. Rec*. **146**: 760-762.

Lopes, M.A., Demeu, F.A., Santos, G.D. and Cardoso, M.G. 2009. Economic impact of calving interval on dairy cattle herds. *Ciência e Agrotecnologia.* **33**: 1908-1914.

Niyas, E. 2017. Ultrasonographic studies on periovulatory follicular dynamics and response to GnRH therapy in repeat breeding cattle with prolonged oestrus. *M.V.Sc. thesis,* Kerala Veterinary and Animal Sciences University, Pookode, 127p.

Omari, S., Vining, L. M. and Wiltbank, M. C. 2020. Effects of progesterone supplementation on follicular development and ovulation in dairy cows. *Reproduction.* **159**: 453–466.

Patel, H. B., Shah, T. M. and Joshi, C. G. 2020. Nanotechnology in livestock reproduction: Current trends and future perspectives. *J. Anim. Sci. Biotechnol.* **11**: 84.

Pacala, N., Corin, N., Bencsik, I., Dronca, D., Cean, A., Boleman, A., Caraba, V. and Papp, S. 2010. Stimulation of the reproductive functions at acyclic cows by Ovsynch and PRID/eCG. *Sci. Papers Anim. Sci. Biotechnol.* **43**: 317-317.

Pawar, S.A., Markandeya, N.M., Sawale, A.G. and Kasbe, R.D., 2012. Studies on induction of oestrus in local anestrus cows with progesterone primed GnRH and progesterone treatment. *Indian J. Anim. Reprod.* **33**: 84-86.

Pursley, J. R. and Martins, J. P. N. 2012. Impact of circulating concentrations of progesterone and antral age of the ovulatory follicle on fertility of high-producing lactating dairy cows. *Reprod. Fertil. Dev.* **24**: 267–271

Ovulatory follicular dynamics after estrus synchronization using intravaginal progesterone implant in dairy cows. (2013). http://i-lib.ugm.ac.id/jurnal/detail.php?dataId=12495

Rajamanickam, T.S.T.M.K. and Kirubakaran, A. 2024. Oestrus induction and fertility response in post-partum anoestrus dairy cows treated with homeopathic medicine. *Ind J. Anim. Sci.* **94** :131-135.

Roche, J.F., Mackey, D. and Diskin, M.D. 2000. Reproductive management of postpartum cows. *Anim. Reprod. Sci.* **60**: 703-712.

Romano, J. E. and Fahning, M. L. 2013. Comparison between 7 vs 9 days of Controlled Internal Drug Release inserts permanency on oestrus performance and fertility in lactating dairy cattle *Italian J. Anim. Sci.* **12**: 63.

Singh, J., Ghuman, S.P.S., Honparkhe, M., Dadarwal, D. and Dhaliwal, G.S. 2012. Risk factors for prolonged estrus in crossbred dairy cattle. *Indian J. Anim. Sci.* **82**: 20.

Stevenson, J.S. 2008. Progesterone, follicular, and estrual responses to progesterone-based estrus and ovulation synchronization protocols at five stages of the estrous cycle*. J. of Dairy Sci.* **91**:4640-4650.

Stevenson, J. S. and Pulley, S. L. 2016. Synchronization strategies for improving reproductive efficiency in dairy cattle. *J. Dairy Sci.* **99**:4961–4974.

Tanaka, T., Arai, M., Ohtani, S., Uemura, S., Kuroiwa, T. and Kim, S. 2008. Influence of parity on follicular dynamics and resumption of ovarian cycle in postpartum dairy cows. *Anim. Rep. Sci.* **108**: 134–43.

Van Werven, T., Waldeck, F., Souza, A.H., Floch, S. and Englebienne, M. 2013. Comparison of two intravaginal progesterone releasing devices (PRID-Delta vs CIDR) in dairy cows: Blood progesterone profile and field fertility. *Anim. Rep. Sci.* **138**: 143-149.

Wiltbank, M. C., Souza, A. H., Carvalho, P. D., Bender, R. W. and Nascimento, A. B. 2011. Improving fertility to timed artificial insemination by manipulation of circulating progesterone concentrations in lactating dairy cattle. *Reprod. Fertil. Dev.* **24**: 238–243. ■