



Effect of progesterone coated nano fibre dermal patch (ProSync-NF) on cattle diagnosed with Anestrus and silent or unobserved estrus

U LAKSHMIKANTAN¹✉, V S VADIVOO², T GEETHA¹ and R MATHIVANAN¹

Veterinary University Training and Research Centre, Tirupur
and

Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, Tamil Nadu 600 051 India

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ABSTRACT

Transdermal Progesterone therapy for the treatment of True and False anestrus was evaluated by using the product ProSync-nano fibre (NF) dermal patch. Eighteen (N = 18) randomly selected animals from different dairy farms during the farm visit by this centre were used for the study. The animals which were reported to be not exhibiting estrus signs even after prolonged postpartum period were designated into three groups. Group I (n = 7) had no palpable ovarian structures treated with ProSync-NF alone, Group II (n = 9) had corpus luteum, treated with ProSync-NF + PGF₂α and Group III (n = 2) animals were diagnosed as having cyst were also treated with ProSync-NF + PGF₂α. Group III animals which were diagnosed with cystic condition did not respond to ProSync-NF therapy. Out of eighteen animals totally used in this study fifteen (83.3%) animals evinced estrus after the treatment with ProSync-NF. Out of fifteen, nine (60%) and six (40%) animals showed estrus around 3 days (≈72 h) and 5 days (≈120 h) after dermal patch removal. Overall, out of the 18 animals treated with 'ProSync-NF' dermal patch, ten cows (55.5%) conceived which indicates fertile estrus induction at the first service. Moreover, the clinical problem like vaginitis would not occur when using dermal route Progesterone therapy. Hence it could be concluded that 'ProSync-NF' dermal patch could be effectively used to induce estrus in anestrus cows.

Keywords: Anestrus, Cows, ProSync-NF dermal patch, Silent, Sub-estrus, Transdermal Progesterone Therapy, Unobserved

In all the reproductive problems in dairy cattle, Anestrus is the most important clinical manifestation that ends up in the substantial losses to the farmers due to reduced calf crop with high treatment cost (Parkinson T J 2019). Anestrus in cattle is generally described as failure of estrus due to functional disturbances in the ovary, many at times characterized by ovary without any of the palpable structures (Ambrose D J 2015, Jena *et al.* 2016, Parkinson T J 2019). In another scenario animal may be regularly cycling but the owner fails to identify the estrus. Occasionally cyst in the ovary may have the symptom of Anestrus. A short period of exposure to high progesterone during the postpartum period is important for expression of estrus and for the subsequent normal luteal function (Ambrose D J 2015). Treating anestrus cows with progesterone increased LH pulse frequency, estradiol concentrations, and LH receptors in granulosa and theca cells of the preovulatory follicle (Rhodes *et al.* 2003). For the treatment of Anestrus, progesterone based therapy could be used with variable results (Kim *et al.* 2004). Usually progesterone therapy is administered through intravaginal

devices (Werven *et al.* 2013) or through progesterone impregnated sponges (Kalyaan *et al.* 2019) inserted into the vagina for a prolonged period of time, usually for seven days. In the present study, Progesterone, the active hormone was attempted to administer through transdermal route. ProSync-NF is a progesterone-based patch that contains progesterone, an active hormone in a nano fibre membrane which releases the hormone for approximately 5 days that mimic the supply of hormone in the blood circulation when affixed over depilated skin of the animal. In general, progesterone-based therapy ensures the occurrence of estrus in animals through sudden withdrawal of the hormone from the peripheral circulation by removing the Progesterone insert or device. In which, a practical question might arise that whether sudden withdrawal of progesterone is possible immediately after the dermal patch removal at the end of the treatment. So, this study was performed to evaluate the response after ProSync-NF therapy in Anestrus, Sub (or) Silent estrus and unobserved estrus.

MATERIALS AND METHODS

Progesterone (approx 1.2 g) coated nano fibre transdermal patch ProSync-NF (Fig. 1) was procured (Quantity 18) from Translational Research Platform for

Present address: ¹VUTRC, TANUVAS, Tirupur 641 604;

²Veterinary University Training and Research Centre, Erode.

✉Corresponding author e-mail: vetlakshmi@gmail.com



- ProSync – NF Progesterone nano fibre transdermal dermal patch fixing in animal
- Picture on the left shows the depilated skin where the Progesterone transdermal patch is placed and fixed using the plaster provided with the product
- Picture in the right shows the affixed transdermal patch over which adhesive plaster is fixed so that the transdermal patch have the proper contact with the depilated skin

Fig. 1. ProSync-NF Progesterone Nano fibre transdermal patch. (a) Product leaflet; (b) Progesterone nano fibre transdermal patch; (c) Glove to handle patch; (d) Plaster to fix the patch in place; and (e) Product carton.

Veterinary Biologicals (TRPVB), a National Accreditation Board for testing and calibration Laboratory (NABL) certified constituent laboratory of Tamil Nadu Veterinary and Animal Sciences University (TANUVAS). This progesterone therapy involves supply of Progesterone through transdermal route through the nanofibre patch affixed over the depilated skin (Fig. 2). All the 18 animals were randomly selected during the farm visits of our centre which were reportedly having the history of not showing any of the estrual signs for the past 3 months to one year and at least calved once. All the animals were subjected for rectal examination to ascertain the reproductive status of the animal and grouped according to the absence of any palpable structure like follicle of Corpus Luteum (Group I) and presence (Group II) of Corpus Luteum (CL) and cyst (Group III). Treatment protocols followed in this study is depicted in Fig. 3. Group I animals which were not having any palpable structures on the ovary were affixed with the ProSync NF dermal patch alone over the depilated skin usually on the rump region (Fig. 2). This patch was allowed

to be affixed for five days and then removed manually as mentioned in the product leaflet. Animals were inseminated with frozen semen on the observed estrus after removal of nanofibre transdermal patch. All the animals were examined after 60 day of post insemination for pregnancy diagnosis. In Group II animals which had corpus luteum and Group III which had cyst were affixed with the ProSync-NF patch and allowed for five days. On the day of transdermal patch removal (Day 5), animals in Group II and III only received a shot of 500 µg of cloprostenol sodium (Inj. Pregma) for the purpose of luteolysis and rest of the procedures were performed as described in Group I. During the study, blood samples were collected for the estimation of serum progesterone on the day of progesterone transdermal patch application (Day 0), on the day of patch removal (Day 5) and on the day of observed estrum (Day AI) after patch removal. Response for the ProSync-NF was presented in percentage and the progesterone levels in the form of Mean $\pm$ SE and analyzed by the student *t* test for any significance between Group I and II.

RESULTS AND DISCUSSION

In the 18 animals examined for the reproductive status seven (38.8%) animals (Group I) had no functional, palpable

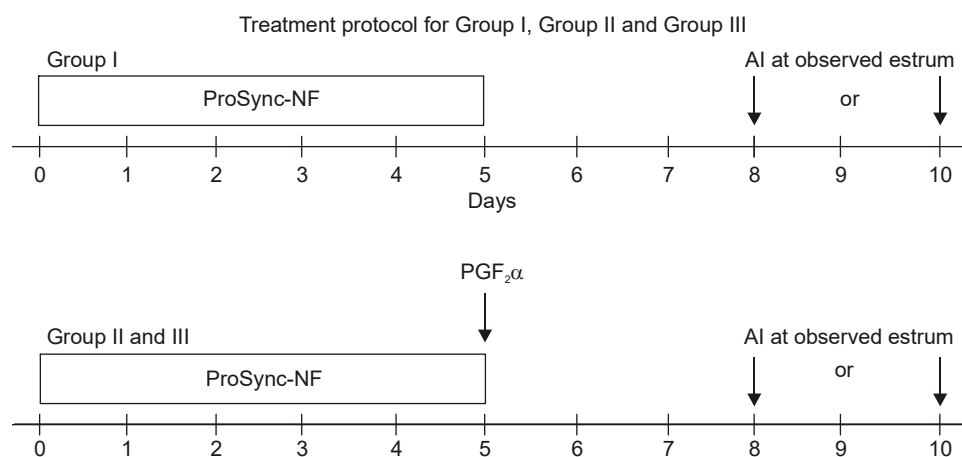


Fig.3. Treatment protocol followed in the study.

structures on the ovary indicating true anestrus nine (50%) animals (Group II) were found with corpus luteum on the ovary. These animals could be in any one of the following categories sub or silent or unobserved estrus (False Anestrus). Remaining two (11.2%) animals were diagnosed with cyst. Animals which had cyst were subjected to reproductive examination ten days later for confirmation. But out of seven animals in Group I, five (71.4%) and two (28.6%) animals were observed in estrus at around 3 days and 5 days respectively after transdermal patch removal and four (80%) animals were pregnant, in the animals, those were evinced estrus around 3 days. These results are comparable with the results obtained by Werven *et al.* 2013, who used intravaginal devices to induce estrus. No animal was pregnant that evinced estrus around 5 days in group I. In Group II, out of nine animal, five (55.5%) and four (44.5%) animals were observed in estrus at around 3 and 5 days; and out of these, four (80%) and two (50%) animals conceived respectively. The percentage of conception is low in the animals which were evinced estrus around 5 days post transdermal patch removal. This could be attributed due to hormonal imbalance that was unable to trigger the production of Gonadotropins at the appropriate time (Parkinson T J 2019).

The level of Progesterone in the circulation on the day of transdermal patch application (Day 0), day of patch removal (Day 5) and on the day of observed estrus (Day AI) after patch removal were tabled in Table 1. From the table it could be clearly understood that the Group I animals categorized into True Anestrus, since no palpable structures like corpus luteum could be identified and also the progesterone level is at basal level. On the Day 5, i.e. on the day of dermal patch removal in Group I, Progesterone level increased as compared with the Day 0, which is comparable as described by Syafnir *et al.* 2011 and Jena *et al.* 2016. Hence it could be inferred that ProSync-NF dermal patch has the effect on increasing progesterone level after affixing it on the depilated skin of the animals under treatment. Post treatment Progesterone level on the day of observed estrus (Day AI) after patch removal was at the basal level in both Group I and II. So, it is understandable that progesterone supplied to the peripheral circulation through transdermal patch was eliminated from the peripheral circulation soon after the removal of patch. Thus, transdermal patch that contains progesterone in the nanofibre membrane could mimic the action of intravaginal drug releasing devices which were used for the same purpose when affixed over the depilated

skin. In the two animals in Group III, those were assumed as follicular cyst and treated using ProSync-NF. But both the animals did not respond to the ProSync-NF treatment. Follicular cysts could be treated with intra vaginal Progesterone releasing inserts with the following hypothesis that increased peripheral concentrations of progesterone result in lowering pulsatile LH secretion and a restoration of the ability of the hypothalamo-pituitary axis to generate an LH surge to estradiol (Parkinson T J 2019). The animals in the Group III were finally diagnosed after Progesterone estimation as luteal cyst, the level of Progesterone was estimated to be 22.1 ng/dl and 14.8 ng/dl respectively. This may be because that the cyst could be of luteinized follicular cyst. Further evaluation and therapy could be attempted by completely luteinizing the cyst; however, these procedures were not carried out because owners of the cows have sold them, once the first attempt of the therapy got failed. However, it is difficult to arrive at any conclusion on the efficacy of ProSync-NF on follicular cyst, since only two animals were found having cyst in the study and both the animals were having high progesterone content in the peripheral circulation.

Anestrus in dairy cows is the most important reproductive disorder and contributes to substantial production loss in terms of number of offspring and number of lactations in the lifespan of the animal due to increased calving interval. Also, the treatment cost to overcome this condition is high. Progesterone based commercially available products like Controlled Internal Drug Releasing Device (CIDR) and similar product TRIU-B and also Progesterone impregnated intravaginal sponges were routinely used for the treatment of Anestrus and also for Synchronization of estrus. In present scenario majority of the progesterone therapy are usually performed through intravaginal route. In such procedures problem of vaginitis were frequently reported (Walsh *et al.* 2008). However, in the present study Progesterone is administered through transdermal route to achieve induction of estrus in Anestrus, sub-estrus or silent estrus cattle. Because this procedure is very simple and the use of transdermal route, complication like occurrence of vaginitis is nullified.

Overall, when we consider the treatment irrespective of the groups, fifteen (83.3%) out of eighteen animals (including the cows with ovarian inactivity) evinced estrus after the treatment with ProSync-NF dermal patch. This percentage is comparable with the results obtained by Walsh *et al.* 2007 and Chebbel *et al.* 2010 as the estrus induction achieved was 84.3% and 85.3% respectively. Out of fifteen, nine (60%) and six (40%) animals showed estrus at around 3 days (≈ 72 h) and 5 days (≈ 120 h) after dermal patch removal, respectively. At pregnancy verification, it was found that ten (66.6%) out of fifteen animals were pregnant. Overall, out of the eighteen animals treated with 'ProSync-NF' dermal patch, ten cows (55.5%) conceived which is indicative of fertile estrus induction at the first service.

Hence, the technology like progesterone dermal patch which is a farmer friendly simple device could be used to

Table. 1. Serum progesterone concentration of ProSync-NF treated animals

Group	Day 0 (Day of Patch application)	Day 5 (Day of Patch removal)	Day AI (Day of observed estrus)
Group I (n = 7)	1.05 ^a ±0.12	3.41 ^a ±0.18	0.62 ^{NS} ±0.07
Group II(n = 9)	4.98 ^b ± 0.39	5.47 ^b ± 0.38	0.70 ^{NS} ±0.08

Different superscript differs significantly (P≥0.05).

induce estrus in Anestrus cattle and may also be used for synchronizing estrus. Also, the results are comparable with the commercially available progesterone releasing devices which are used for the same purpose. Moreover, the clinical problem like vaginitis that could arise because of the insertion of intravaginal devices like CIDR, etc. would not occur when using ProSync–NF for therapy. Hence it could be concluded that ‘ProSync–NF’ dermal patch could be effectively used to induce estrus in anestrus and also in sub or silent or unobserved estrus cows.

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