



## Effects of two different protocols: injection of progesterone vs. injection of GnRH+PGF2a on the onset of estrus in dairy cows with postpartum anestrus

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### ABSTRACT

Postpartum anestrus is one of the most important problems in the dairy industry in the world and especially in Iran. The objective of present study was to compare the efficacy of two hormonal programs for recycling of postpartum anestrus cows. For this reason, 230 Holstein cows from 4 similar dairy herds (with totally 2560 cow and unique management and feeding system) located on the suburb of Tabriz (North-west of Iran) were examined during April 2012 to August 2013. These cows showed no visible oestrus signs until the minimum 60 days postpartum. Clinical examinations were performed twice, with a 10 days interval, and blood samples for progesterone analysis were collected simultaneously with every rectal palpation. Based on progesterone values and clinical examinations only cows with lower progesterone levels ( $P_4 < 2 \text{ ngr/ml}$ ) at both sampling and nonfunctional ovaries (at two consecutive examinations) were randomly allocated into three groups: A, B ( $n=100$ ) and C ( $n=30$ ). Cows in group A were administered a combination of Gonadotropin releasing hormone (GnRH) on day 1 and prostaglandin F2alpha on day 8; cows in group B were administered 125 mg of progesterone for 8 days, whereas cows in the control group C were given placebo. Subsequently all cows were examined for signs of estrus and performed artificial insemination on two consecutive estrus following treatment. In groups A & B the rates of observed oestrus, first service conception rates and second service conception rates were 5% vs. 68%, 60% vs. 25% and 100% vs. 64.7% respectively. In the group of C, cows were not observed in estrus. Significant differences were observed between groups in all fields ( $p \leq 0.01$ ). Thus based on the current study it can be concluded that for recycling postpartum anoestrus cows, progesterone therapy is the treatment of choice.

**Key words:** Anoestrus, dairy cows, GnRH, Postpartum, Progesterone.

### INTRODUCTION

All cows have a period after calving during which they do not show heat. When cows are not coming into heat and ovulating we call them anestrus cows. Anestrus is considered a problem when cows are not seen in heat. Failure to observe heat and heat detection must always be ruled out as the primary problem. Cows in poorer body condition or that are having their first calf (2 year-old cows) are more likely to be anestrus. It is easy to synchronize estrus and AI cows that are cyclic, but more difficult if they are anestrus. In cows with acceptable body condition score ( $\geq 3$ ; 1–5 scale), different natural or synthetic progestogens in combination with other hormones such as eCG, estradiol or GnRH have been used to induce estrus and ovulation in postpartum anestrus cows (Bo and Baruselli, 2002). The physiological goal

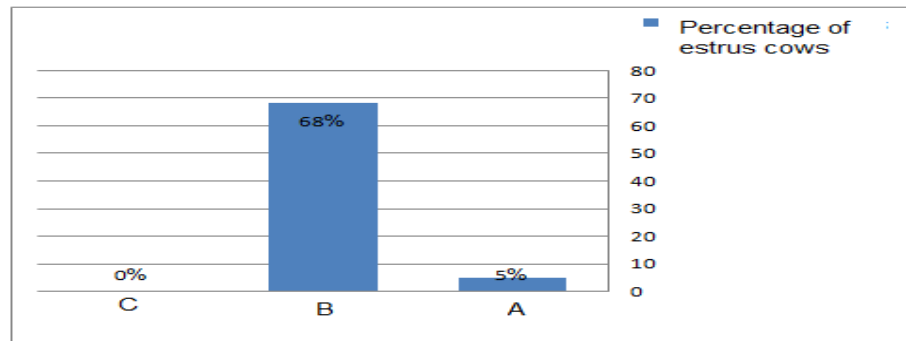
of these methods is to produce an induced corpus luteum (CL) to expose the hypothalamic–hypophyseal ovarian (HHO) axis to greater concentrations of progesterone, similar to natural first postpartum estrous cycles (Yavas and Walton, 2000). Treatment with some of these progestogens have had satisfactory results in crossbred cows under tropical conditions and have been used as an important tool for inducing estrus and ovulation on many farms (Soto et al., 1998; Soto et al., 2002; De Ondiz et al., 2002 and Palomares et al., 2004). For anestrous females, the primary requirement for a successful synchronization system is to induce ovulation and initiate the first postpartum estrous cycle. In the majority of cows with postpartum anestrus, there is no progesterone in the animal's circulation from calving until about day 45. During this time they are not ovulating or forming a CL. The condition worsens when the cow's body condition scores are lesser than 3 (in 1–5 scale). The purpose of the present study was to evaluate the effect of two different hormone treatments (progesterone and GnRH+PGF2 $\alpha$ ) on the onset of estrus in dairy cows with postpartum anoestrus.

### MATERIALS AND METHODS

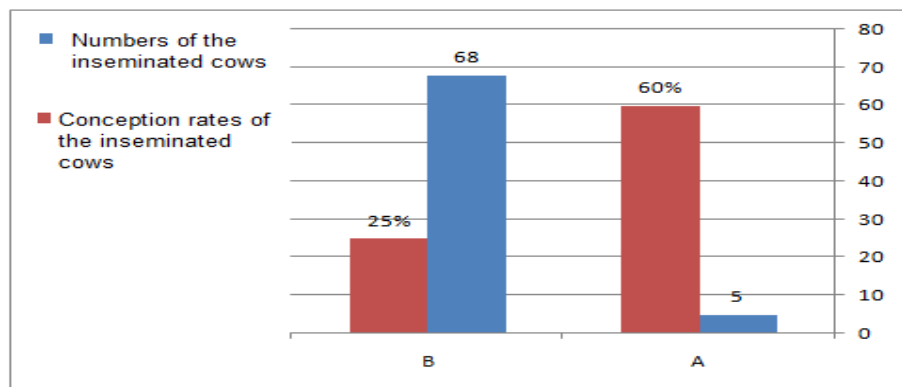
The present study was conducted on the four commercial cattle farms with unique management and feeding systems, which were located in the suburb of Tabriz (north-west of Iran). The farms included a total of 2560 cows and milking was performed three times a day. A total number of 230 anestrous cows were examined during April 2012 to August 2013. Those cows showed no visible oestrus signs until the minimum 60 days of postpartum. In these herds, oestrus detections routinely were carried out visually by an expert technician 4 times/day (8 am, 12, 6pm and 2am) starting on day 45 (Voluntary Waiting Period) postpartum. Clinical examinations were performed twice, with 10 days interval, and blood samples for progesterone analysis were collected simultaneously with every rectal palpation. Progesterone was measured by ELIZA kit (Diaplus Inc., USA). Based on progesterone values and clinical examination only cows with lower progesterone levels ( $P_4 < 2 \text{ ngr/ml}$ ) at both sampling and nonfunctional ovaries (at two consecutive examinations) were randomly allocated into three groups: A&B ( $n=100$ ) and C ( $n=30$ ). In group A, cows received 0.021 mg bucerelin acetate (5 ml Vetocept, manufactured by Abureihan Pharmaceutical Company, Tehran- Iran) on day 1 followed by 25mg dinoprost tromethamine (5 ml Vetalyse, manufactured by Abureihan Pharmaceutical Company, Tehran- Iran) injection on day 8. In group B, all of the cows received 125mg progesterone (5 ml Vetagesteron, manufactured by Abureihan Pharmaceutical Company, Tehran- Iran) intramuscularly for 8 days. In group C, the control group, all cows received 5 ml of saline for 8 days as placebo. Then estrus detection was performed 4 times/day (8 am, 12, 6 pm and 2 am) for the next 5 days.

### RESULTS AND DISCUSSION

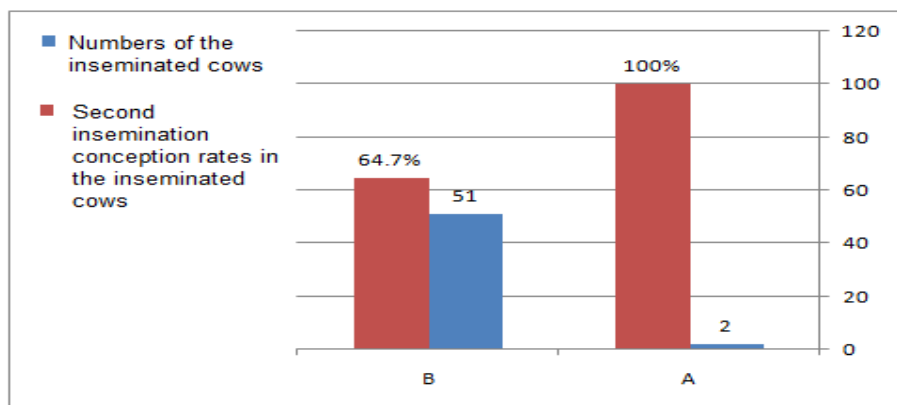
The rates of observed oestrus in groups A, B and C were 5%, 68% and 0%, respectively (Fig.1). The rates of first service conception rate and second service conception rate in the groups of A and B were 60% vs. 25% and 100% vs. 64.7%, respectively (Fig 2 & Fig 3). Comparison of the above results by Chi-square test using SPSS software, version 16 (GraphPad Software, California, USA) showed significant difference observed between the two groups for all observations ( $p \leq 0.01$ ).



**Figure 1.** The rates of observed estrus in groups A (GnRH+PGF2 $\alpha$ ), B (Progesterone) and C (saline) after injecting the hormones.



**Figure 2.** First insemination conception rate in group A (GnRH+PGF2 $\alpha$ ) and B (Progesterone).



**Figure 2.** Second insemination conception rates in group A (GnRH+PGF2 $\alpha$ ) and B (Progesterone).

When a dairy cow is not observed in oestrus by 60 days postpartum, whether actually cycling or not, the condition is defined as Post Partum Anoestrus (PPA). Postpartum anoestrus is a period during which the reproductive axis (hypothalamus-hypophysis-ovary-uterus) recovers after gestation to progressively reach the anatomical and functional status needed to initiate a new pregnancy (Yavas and Walton, 2000). Resumption of cyclic activity after calving is influenced by nutrition, body condition, suckling, lactation, dystocia, breed, age, season, uterine pathology and concurrent disease. In most well managed

dairy herds fewer than 10% of cows fail to ovulate by day 40 post partum. In beef cattle, this may be up to 60% due to the suppressive effect of suckling, nutrition, season etc. Numerous studies in dairy herds have clearly shown that a marked increase in milk production during early lactation increases the incidence of various reproductive problems (Grohn et al, 1994; Macmillan et al., 1996; Poso et al., 1996). Furthermore, the genetic selection for extremely high levels of milk production in dairy cattle, together with changes in their nutritional management and larger herd sizes has also been associated with a gradual decline in fertility. The inability to meet the high energy requirements for both maintenance and production in high yielding cows leads to a negative energy balance, particularly during the first few weeks after calving. Energy balance during the first three weeks of lactation is highly correlated with the interval between calving and first ovulation (Butler et al., 2000). Moreover, it has been reported that severe negative energy balance may prolong the interval between calving and first ovulation. Low energy availability during the first few weeks of lactation impairs LH secretion, but it also reduces the responsiveness of the ovary to LH stimulation (Jolly et al., 1995; Butler 2000). The application of progesterone or synthetic progestin can induce lactating cows and/or synchronizing estrus and ovulation. As a result it is possible to fertilize a large proportion of the herd at the beginning of the period of service, shortening it and reducing the operating costs of artificial insemination (Odde, 1990). Several authors have reported varying effects on fertility after treatment with progestin. Chupin et al. (1975) correlated the shortening of the application of Norgestomet subcutaneous implants from 11 to 9 days with increasing fertility. Results obtained with vaginal sponges suggested that primiparous cows had a lower fertility rate than multiparas, despite having similar time postpartum and body condition (Doray et al., 1991). The use of progesterone or progestagens to treat anoestrus is beneficial because it initiates the oestrous cycle with ovulation, and facilitates the subsequent luteal phase of a normal length. The best results have so far been obtained with the use of progesterone or progestagens, such as norgestomet (Crestar®), combined with an injection of oestradiol at the start of treatment. Injection of eCG (Folligon®) may be used following a period of progesterone treatment, and forms an integral part of the Crestar system to induce oestrus and ovulation in anovulatory anoestrus cows. Using daily transrectal ultrasonography, Rhodes et al. (2000) demonstrated that anoestrus cows treated with small doses of progesterone did not develop persistent ovarian follicles such as those seen in cows treated after oestrous cycles had begun. Therefore it should be possible to obtain satisfactory results in this group of cows with progesterone or progestagen treatment alone. Thus it is concluded that for recycling the ovaries of the postpartum anoestrus cows with low body condition score (<3; 1–5 scale), progesterone therapy is the treatment of choice. Although the first insemination conception rate in progesterone treated cows was lesser than the GnRH treated group (25% vs. 60%), but at the second estrus and AI, the conception rate returned to the normal level (64.7%).

### Acknowledgment

We would like to thank the Research Deputy of The University of Tabriz, for financial support (NO: D/38/3611) of this research.

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