



Application of P4 rapid test and exogenous source of progesterone in prevention of early embryonic death in dairy cows

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Abstract. The objectives of this study were: 1) to determine the percent of cows with low P4 at Day 7-8 post-AI; 2) to treat the animal with low progesterone for 14 days with a P4-releasing intravaginal device (PRID DELTA); 3) to analyze the influence of P4 exogenous supplementation on pregnancy rate in treated and non-treated cows and 4) to create a protocol using "P4 Rapid" test allowing increased pregnancy rate under farm conditions. For this purpose, the current study has been conducted on 155 repeat breeding Holstein cows (<3 artificially inseminated - AI) with prolonged estrus cycle (<26 days). At day 7 and 8 post-AI, the milk progesterone levels were measured (P4 Rapid test). The cows with low P4 (n=104 or 67%) were divided into two groups each n=52. In Group I the progesterone devices were inserted for two weeks and control Group II without progesterone devices. At days 40 and 50 post artificial insemination (AI) all animals (n=104) were submitted to ultrasound pregnancy detection. Pregnancy was registered in 27 animals (51.9%) for Group 1, and in 16 cows (30.7%) for Group II. P4 Rapid is a quick, easy and practically applicable test to measure milk progesterone levels in dairy farms. The method is highly accurate and detects animals with low or high P4 at Day 7-8 after insemination. It also allows identification of conditions such as ovarian cysts, ovarian hypofunction and distinguishing of cycling and non-cycling cows. The combination of P4 measurement by P4 Rapid test and treatment with exogenous P4 to increase circulating concentrations in the first few days after conception can prevent embryo death and improve reproductive efficiency in dairy cattle.

Keywords: cows, P4 rapid, progesterone, PRID DELTA, embryonic death

Introduction

Progesterone (P4) levels are important for a cow's reproductive cycle. At the beginning of oestrus cycle (Day 0 - Day 4) milk progesterone levels are normally below 1.5 ng/ml. During the rest of the cycle P4 levels are greater than 2 ng/ml, depending on physiological status, and sometimes the level can reach 10 ng/ml. After fertilization, the corpus luteum produces progesterone in order to maintain pregnancy. Throughout the pregnancy P4, milk levels rise to 2 ng/ml and 6 ng/ml or more. Later in pregnancy, the placenta and adrenal glands produce extra progesterone. In the days prior to birth P4 levels fall (Foulkes et al., 1982; McNeill et al., 2006).

Several authors (Foulkes et al., 1982; McLeod, 1991) conducted studies on progesterone ability to identify cows returning to service. Those studies detected the cows which had returned to oestrus but were not inseminated. These cows were re-inseminated in first oestrus period, cutting the time between calvings. A weekly sampling from day 35 post-calving as a way to detect whether normal cycling was taking place was described (Foulkes et al., 1982). "P4 Rapid" test will also identify whether a cow has returned to cycling. Perhaps the most important use of progesterone testing, however, is to be certain what the best time is to conduct AI. Daily monitoring of progesterone for 30 days, with insemination on the third day

of low progesterone, led to a 97.5% submission rate and a 62.5% conception rate, compared with 70.7% submission rate and a 47.5% conception rate based on behavioral detection of estrus (Foulkes et al., 1982). Another study, conducted in New Zealand (Dela Rue et al., 2014) used progesterone testing of milk to determine the accuracy in detection of oestrus. A similar study about "P4 Rapid" testing of oestrus and AI was conducted in Serbia (Samardžija et al., 2013). High progesterone levels at oestrus and AI could not result in pregnancy.

Milk P4 testing with Ridgeway Science ELISA has been widely used to study the effect of progesterone levels on the survival of embryo (McNeill et al., 2006) and to study the effect of hormonal treatments from aspects of reproduction (Madgwick et al., 2005).

For the cow, embryonic and fetal mortality rates are about 40% based on a fertilization rate of 90% and an average calving rate of about 55% (Forde et al., 2009); the majority of such loss (70-80%) occurs between Day 8 and Day 16 after insemination (Diskin et al., 2008). During this critical window, the elongating conceptus (embryo and associated extra embryonic membranes) ought to secrete sufficient quantities of interferon tau (IFNT) to signal pregnancy recognition and to prevent the uterine release of luteolytic pulses of prostaglandin F2 α (Spencer et al., 2007). Other studies (Spencer et al., 2007; Diskin et al., 2008) in both beef heifers and dairy cows detected

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positive correlations between naturally occurring elevated concentrations of P4 in the immediate post-conception period, which rise or increase up to Day 7, and the likelihood of conceptus survival and the establishment of pregnancy.

Treatment of exogenous P4 to increase circulating concentrations in the first few days after conception advances post-hatching expansion and elongation of conceptuses in both cattle (Garrett et al., 1988; Carter et al., 2010) and sheep (Satterfield et al., 2006) and is associated with earlier onset of IFNT secretion (Mann et al., 2006).

The aim of the present experiment was to measure ("P4 Rapid" test) progesterone levels in repeat breeding dairy cows at Day 7-8 after AI. If the level appeared to be low, then increase of concentrations of circulating P4 by exogenous supplementation was required. The hypothesis that was set refers to the fact that early embryonic death in most of the cases is caused by low P4 levels. Therefore, the objectives of this study were: 1) to determine the percent of cows with low P4 at Day 7-8 post AI, 2) to treat half of the low progesterone animals for 14 days with P4-releasing intravaginal device (PRID DELTA), 3) to analyze the influence of P4 exogenous supplementation on pregnancy rate in treated and non-treated cows, 4) to create a protocol using "P4 Rapid" test allowing increased pregnancy rate under farm conditions.

Material and methods

This study was carried out in accordance with the European Community Directive 86/609/EC.

Animals

The experiment was performed on 155 repeat breeding Holstein cows (<3 AI) with prolonged oestrus cycle. Non-pregnant cows after three inseminations would be classified as repeat breeders or problem-breeding cows (Casida, 1961). The cows were reared on three dairy farms with the same management and feeding. The farms were selected due to their good reproductive management and proper data collection. The trial was done during spring, summer and fall season. Oestrus was detected by close observation on behavioral signs and electronic activity monitors. Artificial insemination was performed at the exact time according to reproductive management on each farm. The animals were artificially inseminated with semen from a proven sire. Preliminarily, the animals were exposed to ultrasonographic examination for detection of a possible pregnancy.

Experimental design

All examinations were carried out by P4 Rapid test (RIDGEWAY SCIENCE, Gloucestershire, UK). Milk progesterone levels were measured at Day 7-8 post insemination. The milk samples (5 cm³) were taken at any milking before attaching to the milking clusters. The lower test line was the determinant. If there was no progesterone detected, the reagents bind to the test line to produce a strong blue line. The cows determined with "Low" or "Medium" progesterone (n=104) were divided into two groups; Group I, n=52 and Group II, n=52. Group I received immediately

a P4-releasing intravaginal device (PRID DELTA) containing 1.55 g of P4 (CEVA Animal Health Ltd., France) on Day 7-8 of the cycle to increase circulating concentrations of P4 (Atanasov et al., 2014). The P4-releasing intravaginal devices were inserted for 14 days. PRIDs were not inserted in Group II (control group). Thus, the two groups were: 1) Day 7-8 post AI with DELTA, n=52; 2) Day 7-8 post AI without DELTA, n=52 (control).

At Day 40 and 50 post AI, all animals were exposed to trans-rectal ultrasound examination with Aloka SSD 500 Micrus equipment and 5 MHz linear rectal transducer. A positive diagnosis of pregnancy was established when an enlarged uterine lumen with amniotic fluid was seen and the embryo could be visualized.

Statistic

For statistical analysis T-test was used to compare percent at a significant level.

Results

This article covers the challenges of effective bovine fertility management and the value of accurate progesterone monitoring for the effectiveness of hormonal treatments for improving reproductive performances in cows.

The use of "P4 rapid" on the three farms in Bulgaria showed insufficient levels of progesterone on days 7-8 after insemination and increased chance of fetal losses (Table 1). These data showed significant differences ($p < 0.001$) between high and low P4 levels in repeat breeding cows at days 7-8 post insemination (high - only 33% and low - 67%). During the experimental work, some variation in the measured peak levels (intermediate values between 1.5 and 2.5 ng/ml) were established. These intermediate values were considered with "low progesterone". In the present study, P4 supplementation induced changes in pregnancy rate in Group I. Pregnancy rate of 51.9% and 30.7% was found in Group I and Group II, respectively (Table 2). Although the differences were not significant, there is a tendency to increase fertility.

Table 1. Distribution of the milk progesterone levels (P4) at post insemination Day 7-8 in repeat breeding cows

Repeat breeding cows, n (%)	High progesterone (>2.5 ng/ml)		Low progesterone (<1.5 ng/ml)	
	n	%	n	%
155 (100)	51	33 ^a	104	67 ^b

*The percentages in a row marked with different superscript differ at $p < 0.001$

Table 2. Ultrasound pregnancy diagnoses in Group I (experimental) and Group II (control) at Day 50 post-AI

Group	Examined cows (n)	Pregnant (n)	Pregnant (%)
Group I	52	27	51.9
Group II	52	16	30.7

Losses of pregnancy are characterized by early embryonic death, which occurs prior to the period of corpus luteum (CL) maintenance in the cow at days 15-17 of the cycle, and late

embryonic death, which occurs from CL maintenance to the end of the differentiation stage, at approximately 42 days of gestation. After 50 days of gestation, pregnancy losses are less frequent and characterize fetal death. Most pregnancy losses occur prior to the period of maintenance of the CL, but in high producing lactating dairy cattle, substantial losses continue to occur up to 42-56 days after insemination. The steroid hormone progesterone (P4) has a key role in the establishment and maintenance of pregnancy.

Table 3. Diagnoses in non-pregnant cows (Group I and Group II) at Day 50 post-AI

Non-pregnant cows (n)	Diagnoses			
	Ovarian cysts (n / %)	Ovarian hypofunction (n / %)	Corpus luteum persistent (n / %)	Re-inseminated (n / %)
Group I - 25	6/24	8/32	-	11/44
Group II - 36	14/38.8	13/36.2	-	9/25

The results obtained throughout the present study showed that the combined use of P4 measurement ("P4 Rapid") and treatment with exogenous P4 (PRID DELTA) to increase circulating concentrations in the first few days after conception improves reproductive efficiency in dairy cattle.

Discussion

Over the past two decades, numerous studies have been conducted (Spencer et al., 2007; Diskin et al., 2008; Samardžija et al., 2013; Dela Rue et al., 2014) into the use of progesterone (P4) levels as a fertility management tool. In order for the embryo to implant into the uterine wall, the cow must have progesterone levels greater than 5 ng/ml. After fertilization, the rate of a successful pregnancy is determined by the survival of the embryo. Early embryonic death occurs prior to the period of corpus luteum (CL) maintenance in cows at days 13-18 of the reproductive cycle.

The present study revealed the importance of accurate progesterone monitoring for the effectiveness of hormonal treatments for improving reproductive performances in dairy cows. A decrease in circulating progesterone could alter fertility in high producing dairy cows due to P4 involvement in every aspect of reproduction (Sangsritavong et al., 2002). There is an inverse relationship between the level of feed intake and plasma P4 (Rabiee et al., 2001). Several workers (McNeill et al., 2006; Diskin et al., 2008) have indicated that anovulation anestrus, the metabolic status of the animal, some dietary ingredients, as well as the occurrence of diseases, predispose the cows to experience embryonic and fetal death. Although some insemination protocols might impact embryo survival, when timed AI has been implemented properly, it has not influenced embryonic or fetal death in cattle. Improvements in reproductive programs in the future will have to focus on enhancing fertilization rates and minimizing embryonic losses to optimize conception rates in dairy cattle. Insertion of a DELTA PRID on Day 7-8 resulted in a significant increase in P4 concentrations in serum by Day 8 which was maintained until Day 21 (Carter et al., 2008). This window of increased

A diagnosis in non-pregnant cows with low progesterone levels shows (Table 3) ovarian cysts and ovarian hypofunction. During the experimental period, 44% and 25% were re-inseminated (in Group I and Group II, respectively). The reproductive condition in Group I (P4-releasing intravaginal devices) was improved comparing untreated (Group II); cysts 24%: 38.8%; hypofunction 32%: 36.2%; re-insemination in shorter period 44%: 25%, respectively.

P4 corresponds to the period when elevated levels of P4 have been associated with increased embryonic development and survival (Inskeep et al., 2005). Similar to our results where non-pregnant cows with low progesterone levels shows (Table 3) ovarian cysts and ovarian hypofunction, were also published by another worker (Hristova et al., 2014).

Conclusion

P4 Rapid test is a quick, easy and practically applicable test to measure milk progesterone levels on dairy farms. The method is highly accurate and detects animals with low or high P4 at Day 7-8 after insemination. It also allows identification of conditions such as ovarian cysts, ovarian hypofunction and distinguishing cycling and non-cycling cows. The combination of P4 measurement by P4 Rapid test and treatment with exogenous P4 (PRID DELTA) to increase circulating concentrations in the first few days after conception can prevent embryo death and improve reproductive efficiency in dairy cattle.

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