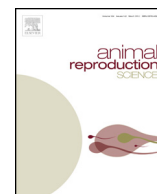




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## Treatment with hCG 4 or 6 days after TAI to improve pregnancy outcomes in repeat-breeding dairy cows



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### ABSTRACT

A study was conducted to evaluate the effect of human chorionic gonadotropin (hCG) administration 4 or 6 days after timed AI (TAI) on P<sub>4</sub> concentration and pregnancy outcomes in repeat breeding dairy cows. All cows were treated by Cosynch protocol before being assigned into 3 groups: CON (*n* = 139): did not receive hormonal treatments; D4 (*n* = 136): received 1500 IU hCG 4 days post TAI; and D6 (*n* = 131): received 1500 IU hCG 6 days post TAI. Plasma P<sub>4</sub> concentration was evaluated on Day of hCG and 12 days post TAI. Pregnancies per AI (P/AI) on Day 45 were greater for D6 (38.9%) than CON (30.9%) groups. Pregnancy losses (PL) were lower for D6 (15.0%) and D4 (26.7%) than CON (37.7%) groups. Treatment by season revealed a greater P/AI and lower PL for D6 (30.3% and 18.2%) and D4 (33.3% and 31.0%) than CON (19.2% and 58.3%) groups during summer, while P/AI was greater for D6 (57.1%) than D4 (30.6%) and CON (37.9%) groups during winter. Treatment by parity indicated a greater P/AI for D4 (46.4%) and D6 (31.6%) than CON (15.8%) groups within primiparous cows. Pregnancy losses were lower for D6 (15.1%) than CON (36.5%) groups within multiparous cows while D4 tended to be lower (13.3%) than CON (50.0%) groups. Plasma P<sub>4</sub> concentration on Day 12 post TAI was higher for treated than for CON groups. Applying hCG 6 days after TAI was beneficial in improving P/AI either in summer or winter as a result of reducing PL and increasing P<sub>4</sub> concentration.

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### 1. Introduction

Repeat breeding is a major problem in dairy cattle leading to large economic loss for dairy producers due to more AI services, increased calving interval and increased culling rates (Bartlett et al., 1986; Lafi et al., 1992). Repeat breeding is defined as the failure of the cow to conceive from 3 or more regularly spaced services in the absence of detectable abnormalities (Zemjanis, 1980). According to Yusuf et al. (2010), the incidence of repeat breeding was

14% in nine commercial herds. The exact cause of repeat breeding in many cases is still an enigma and hence of poor reproductive performance (Yusuf et al., 2010). However, several factors were suggested to be responsible for reduced fertility e.g. nutrition, management practices, genetic, diseases, physiological disturbances, anatomical defects, estrus detection errors, embryonic mortalities and summer heat stress (Badinga et al., 1985; Jainudeen and Hafez, 1993; Thatcher et al., 1994), thereby decreasing overall pregnancy rates (Yusuf et al., 2010; Ferreira et al., 2011). It was suggested that Days 6–8 after insemination is a critical period in repeat breeding dairy cows at which embryo deaths occur (Shelton et al., 1990).

Gonadotropin-releasing hormone (GnRH) has been used over the past 20 years on the day of insemination

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or between Days 11 and 14 after insemination to improve pregnancy rates particularly in repeat breeding dairy cows (Peters et al., 2000). It is well documented that elevation in Progesterone ( $P_4$ ) concentration during the first week of pregnancy plays a major role in reducing embryonic mortalities (Mann and Lamming, 1999; Barnes, 2000) through increasing the secretion of adequate interferon-tau (INF- $\tau$ ) (Mann, 2002). Interferon-tau in turn is very important in extending the lifespan of the corpus luteum (CL) by suppressing estradiol and oxytocin receptor genes (Spencer and Bazer, 1996) and by attenuating the endometrial secretion of  $PGF_{2\alpha}$  (Helmer et al., 1989). Delay in the normal rise in  $P_4$  concentration between Days 4 and 5 post-ovulation and low systemic  $P_4$  concentration during the subsequent diestrus reduces pregnancy rates and results in lower conception rates (Shams-Esfanabadi and Shirazi, 2006). Several approaches were used to increase the concentration of  $P_4$  in early pregnancy in order to reduce the occurrence of embryonic mortalities either by inducing increased endogenous secretion or by administering exogenous  $P_4$ . Administration of GnRH or human chorionic gonadotropin (hCG) after AI can stimulate CL function and/or induce accessory CL formation which in turn increase  $P_4$  and reduce estradiol production, with a consequent positive effect on embryonic survival (Thatcher et al., 2003). Treatment of repeat breeding dairy cows with a combination of estradiol,  $PGF_{2\alpha}$  or GnRH with  $P_4$  supplement was reported to cause elevation in plasma concentration of  $P_4$  and improvement in estrus detection rate for cows that were primed with  $P_4$  than those without  $P_4$  priming with similarity in pregnancy rates (Alnimer and Husein, 2007). Recently, postpartum treatment of dairy cows with hCG 4 days after AI did not significantly increase pregnancy rates on Days 27 and 39 after TAI protocol but reduced pregnancy loss during that period (Fischer-Tenhagen et al., 2010). Santos et al. (2001) reported an increase in pregnancy rates for cows treated with hCG on Day 5 after AI while Hanlon et al. (2005) did not in anestrous cows. However, increased pregnancy rates were observed in embryo transfer recipients treated with hCG on Day 6 after heat (Nishigai et al., 2002). The objective of this study was to evaluate the effect of 1500 IU hCG administration 4 or 6 days after TAI on  $P_4$  concentration at Days 4, 6 and 12 after TAI and pregnancy outcomes (pregnancies per AI and pregnancy losses) in repeat breeding dairy cows.

## 2. Materials and methods

### 2.1. Animals and management

This study was conducted on a commercial dairy farm (Al-Khalidia Modern Cow Farm (Hammoudeh)) in Alkhalidia region northeast of Jordan ( $32^{\circ}33'N$ ,  $35^{\circ}51'E$ ) from January to September 2012. The herd size was around 2500 lactating cows with 1300 milking cows produced around 8230 kg milk/yield Repeat breeders are those cows that were inseminated at least three times and failed to conceive. Cows repeat breeding rates were high among cows in this farm (24% with four or more inseminations and first service conception rate between 30 and 40%). Body

condition was scored on scale from 1 to 5 (1 = emaciated to 5 = extremely fat) at initiation of the study (Edmonson et al., 1989). Cows included in the study (110–140 DIM) were in their 1st to 7th lactation and producing between 26 and 39 kg of milk per day (~9500 kg milk/yield), had at least three unsuccessful inseminations within the current lactation. Cows were housed in free-stall barns provided with shade and were fed a total mixed ration (TMR) of 40% forage (corn silage and alfalfa hay) and 60% concentrate (corn, barley, wheat bran, soybean meal, and commercial concentrate for lactation with trace minerals and vitamins) containing 1.8 Mcal net energy of lactation ( $NE_L$ )/kg and 19% crude protein (CP) at dry matter (DM) basis in accordance with National Research Council (NRC) recommendations (2001). Environmental data for mean maximum temperature ( $35.7^{\circ}C$  and  $23.0^{\circ}C$ ), minimum temperature ( $22.0^{\circ}C$  and  $5.6^{\circ}C$ ), and relative humidity (56.5% and 59.1%) during the experimental period for summer (June to September) and winter (January to May) were obtained from the Official National Station at Dulail area 2 km away from the farm. Cows had ad libitum access to fresh water.

### 2.2. Experimental design

The routine biweekly visit of authors to farm and the local veterinarian examination were used to identify the non pregnant cows about 30 days after the last AI service by scanned ultrasonography (scanner100 Vet; Pie Medical, Maastricht, The Netherlands) using a 7.5-MHz probe as described by Pierson and Ginther (1984). Cows that fit the criteria described above (i.e. at least 3 previous inseminations, 1st to 7th lactation and had normal reproductive tract) were matched for lactation number and number of previous services after each visit. Four hundred and six repeat breeding primiparous ( $n=66$ ) and multiparous ( $n=340$ ) dairy cows which had a functional CL were treated by the same Cosynch protocol [cows were injected with 10  $\mu$ g GnRH agonist (Buserelin, Receptal<sup>®</sup>, Hoechst Roussel Vet GmbH, Wiesbaden, Germany), followed 7 days later by an injection of 25 mg  $PGF_{2\alpha}$  (Lutalyse; Pharmacia & Upjohn S.A.) and terminated by another GnRH injection with TAI 72 h after  $PGF_{2\alpha}$ ]. Thereafter, cows were randomly assigned into three groups: (1) CON ( $n=139$ ): which did not receive any hormonal treatments after TAI; (2) D4 ( $n=136$ ): which received an i.m. injection of 1500 IU hCG (Chorulon, Intervet International, Holland) 4 days post TAI; and (3) D6 ( $n=131$ ): which received an i. m. injection of 1500 IU hCG 6 days post TAI. One experienced AI technician using proven fertility semen (ABS Global, Inc., Deforest, Wisconsin, USA) performed AI throughout the experiment. Semen source was randomized across the treatments. In addition, a routine examination for semen was applied every two months in order to be sure that there is no change in the semen quality. Pregnancy diagnosis was performed using ultrasonography on Day 30 (Pierson and Ginther, 1984) and transrectal palpation of the uterus on Day 45 after TAI.

Pregnancies per AI on Days 30 and 45 post TAI were calculated as the number of cows diagnosed pregnant divided by the number of inseminated cows while pregnancy losses were calculated as the percentage of cows

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