INDUCING OVULATION WITH HUMAN CHORIONIC GONADOTROPHIN IMPROVES CUMULATIVE PREGNANCY RATES OF DAIRY COWS DURING THE WARM SEASON

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Abstract
This study was designed to assess the effects of the GnRH agonist gonadorelin and human chorionic gonadotrophin, during a timed artificial insemination program, on the fertility of lactating dairy cows during the warm and cold seasons of the year. Cows were treated intramuscularly with GnRH-agonist (Day 0) and PGF2α (Day 7), followed by either GnRH-agonist (GPG treatment; 38 animals) or hCG (GPH treatment; 26 animals) on Day 9. All cows were fixed-time inseminated (TAI) 16–22 h after the end of treatment. In this study, replacing the Day 9 dose of GnRH with a dose of hCG may result in an increase in the cumulative pregnancy rate (46.6% vs. 25%, $P \leq 0.05$) but not the pregnancy rate (26.6% vs. 18.8%, $P > 0.05$) in heat-stressed cows ($THI > 70$). However, in cold season, no effects were observed in the pregnancy rates or cumulative pregnancy rates of the examined cows. This finding indicates that hCG therapy in TAI programs may mitigate the negative effects of heat stress on dairy cows reproduction. This tendency can be explained also by some studies that have shown that hCG can significantly reduce the number of small luteal cells while increasing the number of large luteal cells as the corpus luteum gets bigger in diameter, area, and volume.

Key words: hCG, TAI, pregnancy rate, heat stress, dairy cows.

INTRODUCTION

The dairy sector has created and extensively implemented timed artificial insemination (TAI) programs to get over the limitations of estrus detection and enable scheduled artificial insemination (Caraviello et al., 2006; Pursley et al., 1995). However, between 40 and 60 percent of the treated animals do not conceive at the time of TAI. The failure of TAI could be caused by a certain number of factors.

For exemple, 12–21% of cows given a single dose of PGF showed incomplete luteal regression in a Ovsynch protocol (Wiltbank et al., 2015; Brusveen et al., 2009; Carvalho et al., 2015). A single dose of prostaglandin F2α (PGF) cause incomplete luteal regression which is the source of increased progesterone (P4) concentration at the second GnRH treatment.

In cows that began a TAI program in a low P4 condition, the subsequent decline in pregnancy per AI (P/AI) may be significant (Giordano et al., 2012; Carvalho et al., 2015; Santos et al., 2016). In addition, increasing P4 at the beginning of the treatment has a favorable influence on the growth of the dominant follicle and luteal regression, hence improving fertility (Bisinotto et al., 2011; Pursley et al., 2011; Borchardt et al., 2018). Another factor which cause failure of TAI protocol is low circulating P4 after breeding which may impact embryo development and maternal recognition of pregnancy (Mann and Lamming, 2001). Large luteal cells are more widespread after human chorionic gonadotrophin (hCG) therapy in cows (Helmer and Britt, 1987; Sianangama and Rajamahendran, 1992) which is also associated with a decrease in smaller luteal cells and an increase in plasma P4 levels (Breuel et al., 1989; Fricke et al., 1993; Diaz et al., 1998). In some studies, hCG is administered as part of the Ovsynch protocol to synchronize and induce ovulation (De Rensis et al., 2002; Schmitt et al., 1996) in order to improve fertility. According to Kinser et al. (1983), in contrast to GnRH, hCG has a longer half-life than natural LH and works independently of the pituitary gland.

Thus, the aim of the present study was to evaluate the effects of hCG administration within an Ovsynch protocol on the fertility in lactating dairy cows during the warm and cold seasons.

MATERIAL AND METHOD

This study was focused on a Holstein-Friesian dairy herd located in the North-Eastern part of Romania. During the studied period, the average number of lactating cows in the herd was approximately 340, and the average annual milk production per cow was about 8,000 kg milk/305 days. According on their body condition score and whether or not they were diagnosed as being pregnant with twins, dry cows were housed in a separate group and transferred 21 days prior to birth to the "parturition group" (close-up group). The cows were housed in free-stall barns with concrete floors and a straw bed, their feeding being based on a Total Mixed Ration twice daily with *ad libitum* access to water, according on their milk production and cow size. In order to maintain the animals health, standard management procedures were used.

In order to reduce variation, only animals that corresponded in terms of a good health and physical condition at the beginning of treatments were chosen. Thus, only cows scoring between 2.5 and 3.5, which were regarded to be in a good body condition, were included in the trial (Gearart et al., 1990). Regarding the voluntary waiting period, a minimum of 60 days after parturition have been set.

Estrus cows were detected from the AfiMilk (AfiMilk, Kibbutz Afikim, Israel) estrus daily report and each animal was examined by an experienced veterinarian. The indications of estrus signs were represented by the desire to mount other cows, chasing herd members, restlessness, chin resting, sniffing herd mates vagina and bellowing, congestion, relaxation, and mucus secretion from the vulva. The behavior of standing to be mounted was regarded as an indication of real estrus signs.

Two groups of milking cows were organized to conduct this study, as following: **GPG group** (38 dairy cows): (Day 0) GnRH (Gonavet Veyx, Veyx-Pharma GmbH, Germany, 100 micrograms of Gonadorelin[6-D-Phe], i.m.) followed by PGF (PGF Veyx Forte, Veyx-Pharma GmbH, Germany, 0.5 mg cloprostenol/cow, i.m.) 7 days later and then a second GnRH dose 36 h after the PGF and AI after 16h. The second group was presented by **GPH group** (26 animals): (Day 0) GnRH (Gonavet Veyx, Veyx-Pharma GmbH, Germany, 100 micrograms of Gonadorelin[6-D-Phe]/cow, i.m.) followed by PGF (PGF Veyx Forte, Veyx-Pharma GmbH, Germany, 0.5 mg cloprostenol/cow, i.m.) 7 days later, and then hCG (1500 IU, Chorulon, MSD, Holland) given 36 h after the PGF and AI after 16h.

For all the animals included in the study, AI was performed by two operators with over 20 years of experience. The pregnancy diagnosis was performed with the Draminski iScan2 veterinary ultrasound instrument (Draminski S.A., Poland).

Statistical analysis

Depending on the temperature humidity index (THI), we established two distinct climate periods in the studied area: warm (May to September, THI exceed 70) and cold (October to April, THI below 70). Thus, all the data regarding the performed treatments were used to compare the effect of treatments on reproductive success during warm and cold periods. To determine the pregnancy rate at TAI, we evaluated the percentage of animals among the total number of cows in the relevant group that became pregnant after receiving TAI. The cumulative pregnancy rate was calculated as the proportion of cows in each group who got pregnant after two sessions of artificial insemination (first AI plus return AI) within 30 days of the completion of treatment.

The comparative statistical analysis between GPG and GPH groups in terms of pregnancy rate and cumulative pregnancy rate was performed by applying the Chi-square test (Thrusfield, 1995). The level of statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSIONS

Table 1 highlights the reproductive performance of dairy cows subjected to the GPG and GPH protocols during the heat stress period compared to non-heat stress periods of the year. It was observed that in TAI programs, the pregnancy rates for the two groups of cows in heat stress periods did not show significant differences compared to periods with THI < 70. However, a significant difference between the two protocols was observed ($P < 0.05$), in terms of the cumulative pregnancy rate during the heat stress period (THI > 70).

Regarding TAI programs, replacing the day 9 dose of GnRH with a dose of hCG may result in an increase in the cumulative pregnancy rate in stressed heat cows (THI > 70). This indicates that hCG treatment can counteract the detrimental effects of heat stress on the reproduction of dairy cows. This can be explained by the fact that, according to some studies, hCG has the ability to cause a significant decrease in the number of small luteal cells (Farin et al., 1988; Helmer et al., 1992), while the number of large luteal cells increases proportionally with the corpus luteum's diameter, area, and volume (Rajamahendran and Sianangama, 1992; Santos et al., 2001). All of these actions serve to promote dairy cow fertility by boosting luteal activity and, consequently, serum progesterone levels (De Rensis et al., 2008). In a study conducted by De Rensis et al. (2002), it was observed that the hCG product administered in OvSynch programs is less successful than the GnRH product. This can be explained by the fact that the animals included in our study, as in the study carried out by De Rensis et al., (2008), were selected to be part of the two groups only if by ultrasound examination, the presence of a large follicle and a corpus luteum in the structure of the ovaries at the beginning (day 0) of the GPG and GPH protocols were detected.

Table 1

Pregnancy rate at TAI and cumulative pregnancy rate in dairy cows after applying the protocol GPG (GnRH on day 0, PGF2α on day 7, GnRH on day 9 and AI at 16h) and GPH (GnRH on day 0, PGF2α on day 7, hCG on day 9 and AI at 16h)

Reproductive parameters	THI	**Group GPG**	**Group GPH**	**Statistical significance**
Pregnancy rate at TAI	< 75	50% (11/22)	54.5% (6/11)	P > 0.05
	> 75	18,8% (3/16)	26.6% (4/15)	P > 0.05
Cumulative pregnancy rate	< 75	72.7% (16/22)	81.8% (9/11)	P > 0.05
	> 75	25% (4/16)	46,6% (7/15)	P ≤ 0.05

CONCLUSIONS

The cumulative pregnancy rate during the heat stress period is significantly increased when hCG is used in TAI programs, assuming that females undergoing these protocols have developed follicles and the corpus luteum at the time when protocol was initiated.

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