IMPROVING FERTILITY IN MULTIPAROUS COWS BY INDUCING OVULATION WITH GNRH AGONIST - GONADORELIN OR HUMAN CHORINIC GONADOTROPHIN

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Abstract
This study was designed to assess the effects of the GnRH agonist gonadorelin and human chorionic gonadotrophin administered at first artificial insemination on the fertility of lactating dairy cows. The lactating dairy cows were divided into three groups: the hCG-group consisting of primiparous and multiparous cows that expressed the specific behavior of estrus with AI 12 hours after the end of estrus along with administration of 1500 IU of human chorionic gonadotropin (hCG); the GnRH-group consisting of primiparous and multiparous cows that showed the specific behavior of estrus with AI 12 hours after the end of estrus along with administration of 2 mL of Gonadorelin; and the control group (C-group) consisting of primiparous and multiparous cows that showed the behavior specific to estrus and that were artificially inseminated (AI) 12 hours after the end of estrus signs without any treatment. According to our results, administering GnRH agonist or hCG products to multiparous cows in order to induce ovulation increases the pregnancy rate during the first artificial insemination (46.6%, 57.1% vs. 22.2%, $P \leq 0.05$). Both products don't have any effects on primiparous dairy cows (45%, 42.9% vs. 50%, $P > 0.05$).

Key words: GnRH agonist, hCG, dairy cows, ovulation, multiparous.

INTRODUCTION

More commonly, producers face the issue of infertility in dairy cows. In spite of numerous research on the topic, the reproductive efficiency of dairy cows has recently declined. This downward tendency is primarily due to the low rate of oestrus identification and ovulation timing. In the past, it was assumed that the low rate of oestrus detection was due to the farmer's lack of interest because of other farm priorities (Alexander et al., 1984). As research on this area has developed, it has become clear that a number of farm-level parameters have a significant effect on the rate of estrus detection and, implicitly, the timing of ovulation (Dransfield et al., 1998; Stevenson et al., 1998; Van Vliet and Van Eerdenburg, 1996).

Artificial insemination (AI) is regarded as the most effective approach for achieving high pregnancy rates in dairy cows, and the behavior of other cows accepting to be mounted is considered to be a specific indicator for determining the optimal time to do AI. (Bostedt, 1976; Maatje et al., 1997; Nebel et al., 2000; Trimberger, 1948; Walker et al., 1996).

Very few studies have examined the effect of hormone therapy for ovulation management in dairy cows on reproductive performance. According to Chenault et al. (1990), a single dose of the GnRH analog buserelin induces the release of LH at the pituitary level for about 5 hours, which is approximately half the duration of the hormone's normal release from the pituitary level (Rahe et al., 1980). This phenomenon can interfere with both ovulation and the subsequent development of the corpus luteum.

Human chorionic gonadotropin is an additional hormone that may be used in ovulation induction methods (hCG). It appears that hCG-based products are more effective than GnRH-based treatments because they indirectly improve luteal function, embryo viability, and fertility (Schmitt et al., 1996, Mee et al., 1990; Stevenson et al., 1990; Khan et al., 2003). However, it is widely acknowledged that hCG and GnRH products have similar effects on ovarian function (Rajamahendran and Sianangama, 1992; Fricke et al., 1993), with the specification that hCG operates independently on the pituitary gland and has a longer duration of action than spontaneous LH discharges (Kinser et al., 1983).

Thus, the objective of this study was to examine the ovarian structures using ultrasound during dairy cows' estrous behavior in connection to parity-based pregnancy rates. In addition, the influence of GnRH and hCG-induced ovulation on pregnancy rates was assessed.

MATERIAL AND METHOD

Northeastern Romanian Holstein-Friesian dairy herd served as the subject of this study. The average number of lactating cows in the herd was 340 during the study period, and the average annual milk output per cow was around 8000 kg milk/305 days. Depending on their body condition score and whether or not they were pregnant with twins, dry cows were kept in a separate group and moved to a "parturition group" 21 days before the parturition (close-up group). The cows were housed in free-stall barns with concrete floors, straw bed and fed a Total Mixed Ration twice per day with *ad libitum* water access, according to the level of milk production and cow size. To keep the animals healthy, standard management practices, were used.

All ovarian follicles with a diameter of less than 5 mm were measured by transrectal ultrasonography examination (Draminski iScan2 veterinary ultrasound, Draminski S.A., Poland) to evaluate the size of the mature ovarian follicle, close to ovulation. The follicle with a diameter higher than 10 mm and revealing at least 2 mm more than the other follicles was referred to as the dominant (mature or preovulatory) follicle (Sirois and Fortune, 1990).

A minimum of 60 days following calving have been designated as the voluntary waiting period.

The AfiMilk (AfiMilk, Kibbutz Afikim, Israel) estrus daily report was used to identify cows in estrus, and each one was examined by an experienced veterinarian. The indications of estrus included attempts to mount other cows, chasing herd mates, restlessness, chin resting, sniffing herd mates' vagina and bellowing, congestion, relaxation, and mucus discharge from the vulva. The presence of standing estrus was considered to be a sign of true estrus.

Three groups of milking cows were organized to conduct this study: control group (group M) consisting of primiparous and multiparous cows that showed the specific behavior of estrus and that were artificially inseminated (IA) 12 hours after the end of estrus; GnRH group consisting of primiparous and multiparous cows that showed the specific behavior of estrus, AI 12 hours after the end of estrus, with the administration of 2 ml of Gonadorelin (Gonavet, VEYX PHARMA GmbH, Germany) and the hCG group consisting of primiparous and multiparous cows that showed the specific behavior of estrus, AI 12 hours after the end of estrus signs with the administration of 1500 IU hCG human chorionic gonadotropin (MSD, Animal Health, USA). For all cows included in the study, AI was performed by two operators with over 20 years of experience. The pregnancy diagnosis was performed using the Draminski iScan2 veterinary ultrasound (Draminski S.A., Poland).

Statistical analysis

The comparative statistical analysis between control and experimental groups in terms of conception and pregnancy rates was performed by applying the Chi-square test (Thrusfield, 1995). The comparative evaluation (primiparous vs. multiparous) of the intervals until the first detected estrus, of the voluntary waiting periods, the Interval between artificial inseminations (postpartum days, mean ± SD) was performed by applying Mann's U test-Whitney. The level of statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSIONS

Table 1 displays the reproductive parameters associated with the ovarian activity of the dairy cows on the studied farm. Multiparous cows have a longer interval between their first detectable estrus compared to primiparous cows (P 0.05). We can therefore hypothesize that uterine involution in multiparous cows requires a longer postpartum period than in primiparous cows. However, this aspect had no effect on the postpartum interval to the administration of the first dosage of semen in primiparous cows compared multiparous cows (Table 1, $P > 0.05$).

After performing the pregnancy diagnosis, it was observed that the conception rate of primiparous cows did not differ significantly from that of multiparous cows, with values of 47.5% for primiparous cows and 39% for multiparous cows.

In order to continue evaluating reproductive activity, the interval between estrus was determined in non-pregnant cows that received a second or third dose of semen to increase the proportion of pregnant cows on the farm. Therefore, in this study, the interval between estrus signs tended to be longer for multiparous cows than for primiparous cows (Table 1).

In a study conducted by Remnant et al. (2014), the interval between estrus was analyzed, the results revealing that this parameter has a tendency to increase with parity.

Withal, Diskin et al. (2011) observed that increased values of the intervals between the first and second AI and between the second and third AI were associated with the presence of early embryonic death. Current studies on this topic indicate that early embryonic death is higher in multiparous cows compared to primiparous cows (El-Tarabany et al., 2016). The explanation for this is given by Kafi et al. (2015), who found that dairy cows achieve excessive body condition score losses, and this fact causes a disruption of the length of the luteal phase and implicitly of

maintaining the functionality of the corpus luteum after calving.

The results of this study are presented in table 2. Thus, in this study, in primiparous cows, the pregnancy rate at the first artificial insemination does not show differences ($P > 0.05$) between the three groups of cows.

However, in the case of multiparous cows, the pregnancy rate at the first AI was significantly higher ($P \leq 0.05$) in the GnRH and hCG groups compared to the M group. In addition, in group M, it was observed that there was a difference (50% vs. 22.2%, $P = 0.001$) in the first AI pregnancy rate between primiparous cows compared to multiparous cows (Table 2).

CONCLUSIONS

In conclusion, the administration of hormonal products based on GnRH and hCG at the time of AI can improve the pregnancy rates at the first AI in multiparous cows.

Table 1

Reproductive indicators of dairy cattle in selected dairy farms

Reproductive parameters	Primiparous (n = 158)	Multiparous (n = 182)	Total (n = 340)
First detected estrus (days postpartum, M ± SD)	50 ± 19.2[b]	71 ± 14.3[a]	66 ± 19,1
Adjusted voluntary waiting period (days postpartum, M ± SD)	77.75 ± 42.23	82.29 ± 37.19	79.95 ± 39.84
Conception rate (%)	47.5 %	39 %	42.9 %
AI1 – AI2 interval (days postpartum, M ± SD)	38.61 ± 23.28	45.67 ± 27.38	42.3 ± 25.66
AI2 – AI3 interval (days postpartum, M ± SD)	34.11 ± 19.65	40 ± 21.3	36.78 ± 20.52

AI1 - first artificial insemination; AI2 - second artificial insemination; AI3 - third arificial insemination; M ± SD - mean ± standard deviation. [a,b] Values with different superscripts within rows denote significant differences between groups detected by the Mann-Whitney test (a vs. b, a > b, P < 0,05), M ± SD = mean ± standard deviation.

Table 2

Ovulatory response of dairy cows in experimental groups subjected to treatment with (GnRH, hCG) compared to group M (no treatment). Cows in the experimental groups were given single doses of GnRH and hCG at the time of AI

Items	Group M		Group GnRH		Group hCG	
	Primiparous (n = 55)	Multiparous (n = 73)	Primiparous (n = 60)	Multiparous (n = 43)	Primiparous (n = 34)	Multiparous (n = 33)
Ovulatory follicle diameter (mm, M ± SD)	16.04 ± 5.39	15.29 ± 4,33	16.46 ± 4.03	17.55 ± 4.2	17.27 ± 5.69	15.69 ± 5
Pregnancy rate at first AI (%)	50[d] (9/18)	22.2[b,c] (6/27)	45 (9/20)	46.6[a] (7/15)	42.9 (6/14)	57.1[a] (8/14)
Pregnancy rate at two and more artificial inseminations(%)	35.1 (13/37)	32.6 (15/46)	42.5 (17/40)	33.3 (9/27)	40 (8/20)	31.6 (6/19)

[a,b] Values with different superscripts within rows denote significant differences between groups detected by the chi-square test (a vs. b, a > b, P<0.05); [c,d] Values with different superscripts between primiparous and multiparous cows; M ± SD = mean ± standard deviation.

ACKNOWLEGMENTS

The research reported in this publication was supported by the Romanian Ministry of Agriculture and Rural Development by the research grant ADER 8.3.4/2019.

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