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Comparative Study of Resynchronization Conception Rate Based on Ovsynch 48 and 56 Hour in Dairy Cow

[1, 2]A. Bahrami, [3]S. Mosaferi, [4]H. Hamali and [5]Z. Ostadi
[1]Young Researchers Club, [2]Department of Veterinary Medicine,
[3]Department of Clinical Science, Faculty of Veterinary Medicine,
Tabriz Branch, Islamic Azad University, Tabriz, Iran
[4]Department of Clinical Science, Faculty of Veterinary Medicine,
University of Tabriz, Tabriz, Iran
[5]Department of Anesthesiology, Faculty of Medicine,
Tabriz University of Medical Science, Tabriz, Iran

Abstract: The objective of this study was to compare two strategies for resynchronization of ovulation based on ovsynch 48 and 56 h in non-pregnant dairy cow diagnoses using trans rectal ultrasonography Lactating Holstein cows (n = 150) were submitted for postpartum Timed Artificial Insemination (TAI) using a Ovsynch 48 and 56 h protocol. After diagnosed non-pregnant, cows were randomly assigned to initiate resynchronization. Non-pregnant cows as determined by ultrasonography on day 30 post-AI and were divided into 3 groups. Cows in 1st group receiving an injection of PGF2α in 30 days and detected in estrus were inseminated and 2nd group receiving an injection of PGF2α 7 days after the initial GnRH injection and a 2nd GnRH injection 48 h after the PGF2α injection and 3rd group receiving an injection of PGF2α 7 days after the initial GnRH injection and a 2nd GnRH injection 56 h after the PGF2α injection and cows in both treatments were inseminated approximately 16-20 h after the 2nd GnRH injection. Therefore, pregnancy rates were evaluated for 3 groups that 1st group (n = 50) 18 pregnant and 32 non-pregnant, pregnancy rate in this group 36% and cows in the group 2 (n = 50) 11 pregnant and 39 non-pregnant, pregnancy rate in this group 22% and cows in the group 3 (n = 50) 16 pregnant and 34 non-pregnant, pregnancy rate is 32% and pregnancy rate between group 1 and 2 was significant p = 0.0044 ($p<0.05$), pregnancy rate between group 2 and 3 was significant too p = 0.0446 ($p<0.05$) and pregnancy rate between group 1 and 3 was not significant p = 0.5202 ($p>0.05$).

Key words: Resynchronization conception, ovsynch, dairy cow, injection, Tabriz

INTRODUCTION

Because shortened duration and decreased expression of estrus in high-producing dairy cows present a challenge for detection of estrus (Britt, 1995), accurate and early detection of non-pregnant cows combined with a program for resynchronization of ovulation will improve reproductive efficiency of dairy herds. Programming the stage of the estrous cycle for resynchronization after the initial TAI is a physiological approach to reducing the interval between TAI services (Bartolome *et al*., 2005a, b). Cows diagnosed non-pregnant after a Presynch + Ovsynch and TAI should be resynchronized as early as possible when diagnosed non-pregnant. Protocols for resynchronization of ovulation (Resynch) can increase the effective AI service rate and reduce the interval between AI services (Fricke *et al*., 2003).

One approach to Resynch strategies is to reduce the interval between TAI by choosing the optimal stage after TAI to initiate Resynch (Fricke *et al*., 2003). Protocols for resynchronization of ovulation (Resynch) can increase the effective AI service rate and reduce the interval between AI services (Fricke *et al*., 2003). Assigned cows to protocols for resynchronization according to stage of the estrous cycle based on ultrasound and palpation 30 days after AI (Ultrasonography enables early pregnancy diagnosis and detection of non-pregnant cows). An alternative approach is to time initiation of Resynch at an interval after initial TAI when a high proportion of cows would be expected to be day 5-12 of the estrous cycle similar to that achieved using Presynch + Ovsynch. Because PR/AI to Resynch was poor for cows without a Corpus Luteum (CL) at the 1st GnRH or PGF2α injections of Resynch (Fricke *et al*.,

Corresponding Author: S. Mosaferi, Department of Clinical Science, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran

2003) alternative treatments aimed at improving fertility of cows lacking a CL at initiation of Resynch may further improve an overall resynchronization strategy. Conception rate to 1st postpartum TAI is around 35% (Fricke *et al.*, 2003), resulting in a large proportion of non-pregnant cows that need to be resynchronized after their 1st postpartum TAI. Many dairy producers have used Ovsynch for resynchronizing ovulation (i.e., Resynch) for cows diagnosed not pregnant to a previous TAI, however the optimal timing of this strategy has not been well established.

One strategy used to increase conception rate to TAI after a Resynch protocol has been to choose intervals after TAI to initiate the Resynch protocol based on assumptions regarding the physiology of the estrous cycle (Fricke *et al*., 2003).

The development of hormonal synchronization protocols that allow for Timed AI (TAI) have provided a management tool for initiating 1st postpartum AI and thereby precisely controlling the voluntary waiting period in lactating dairy cows. A common hormonal protocol for synchronizing ovulation in lactating dairy cows uses injections of GnRH and PGF2α (Burke *et al*., 2000) and is an effective method for hormonally programming cows to receive TAI.

Protocols for resynchronization of estrus that considered the stage of the estrous cycle have been applied after detection of non-pregnant cows by per rectum palpation of the uterus and assignment of protocols based on the presence or absence of a CL (Bartolome *et al*., 2005a, b). Another approach for resynchronization after non-pregnancy diagnosis by ultrasonography was either to initiate the Ovsynch protocol 7 days before ultrasonography (Chebel *et al*., 2003) or to take advantage of a natural resynchronization after previous service with application of shortened protocols that used PGF2a to induce luteolysis and then GnRH (Stevenson and Martel, 2009) or ECP (Chebel *et al*., 2003) to induce ovulation. Ovsynch synchronizes follicular development, luteal regression and ovulation such that artificial insemination can be conducted at a fixed-time without the need for estrus detection. Further, giving the 2nd GnRH injection at 56 h after PGF2α in the Ovsynch protocol, followed in 16 h by timed AI has produced more P/AI in 1st and repeat-service dairy cows (Brusveen *et al*., 2008). Most herds initiate the Ovsynch program [injection of Gonadotropinreleasing Hormone (GnRH) 7 days before and 48 h after Prostaglandin F2α (PGF2α) with a fixed-time AI administered between 16 and 24 h after the 2nd GnRH injection] 7 days before pregnancy status is diagnosed. Resynchronization can then be completed with reinsemination 72 h after a non-pregnancy status is confirmed. Precise follicular maturation and its synchronization with the demise (luteolysis) of the corpus luteum or corpora lutea (Stevenson and Martel, 2009).

Initiating the 1st GnRH injection of Resynch 1 week before pregnancy diagnosis can further reduce the interval between TAI. The optimal time to initiate synchronization using Ovsynch for 1st TAI occurs between day 5 and 10th of the estrous cycle (Moore *et al*., 2005; Gumen *et al*., 2003).

These reports on the effect of timing of the 2nd GnRH injection relative to PGF2α and AI are inconsistent. Detection of estrus is one of the major challenges in the reproductive management of dairy cows (Britt, 1995).

Highly synchronous returns to estrus and overt estrous behavior would facilitate reinsemination of cattle non-pregnant to Timed Artificial Insemination (TAI). Based on the results of previous resynchronization studies, synchrony of behavioral estrus and the proportion of non-pregnant cattle detected in estrus must be improved. However, treatments to synchronize ovarian follicular development and increase estrus detection rates must not jeopardize pregnancy to TAI.

MATERIALS AND METHODS

Cows, housing and diets: The study was conducted from April to December, 2011 in Azerbaijan (Iran) Lactating Holstein cows (n = 150) from a commercial dairy farm with 1000 lactating dairy cows and a daily milk production average of 31 kg. Cows were housed in free-stall barns and individual pens were virtually identical in design, size and number of cows housed. Of the 150 lactating dairy cows initially enrolled in the study, 75 were removed because of poor udder conformation (n = 17), uterine and ovarian adhesions (n = 9) and death (n = 36) or were sold (n = 13) before the end of the voluntary waiting period on study day 49. Therefore, a total of 977 cows were used for statistical analyses. Cows were fed two different diets as TMR according tothe stage of lactation with an immediate postpartum diet fed between 1 and 21 DIM and a lactating diet for the remainder of lactation. Cows were fed twice daily and diets were based on corn silage, alfalfa hay, soybean meal, corn, oat cottonseed meal, calcium salts of palm oil and a mineral, vitamin. The 2 diets were designed to meet or exceed NRC requirements for lactating Holstein cows weighing 650 kg and producing 45 kg of milk day^{-1} containing 3.5% fat.

Study design: On day 0, non-pregnant cows (n = 150) were divided randomly into 3 groups.

Cows in the group 1: Cows diagnosed non-pregnant on day 0 received 25 mg IM of PGF2α (5 mL of Lutalyse1 sterile solution; Pfizer Animal Health, New York, USA) and insemination in observation estrus (Detected in estrus inseminated).

Cows in the group 2: Cows diagnosed non-pregnant on day 0 received 100 mg IM of GnRH (2 mL of Cystorelin 1; Merial Ltd., Iselin, NJ, USA) received 25 mg IM of PGF2α on day 7 after 48 h received 100 mg IM of GnRH (2 mL of Cystorelin 1; Merial Ltd., Iselin, NJ, USA) and TAI 16-20 h later).

Cows in the group 3: Cows diagnosed non-pregnant on day 0 received 100 mg IM of GnRH (2 mL of Cystorelin 1; Merial Ltd., Iselin, NJ, USA), received 25 mg IM of PGF2α on day 7 after 56 h received 100 mg IM of GnRH (2 mL of Cystorelin 1; Merial Ltd., Iselin, NJ, USA) and TAI 16-20 h later.

Determination of stages of the estrous cycle, ovarian cysts or anestrus: On day 0, cows were classified according to different stages of the estrous cycle or presence of ovarian cysts or anestrus combining per rectum examination and Ultrasonography of the genital tract to determine ovarian structures and uterinecharacteristics (Table 1). Detection of a functional CL included morphology at palpation (i.e., line of demarcation and distortion in the shape of the ovary) and visualization atultrasonography. A valid criticism for diagnosis of a functional CL is that plasmaprogesterone was not determined, therefore there could be a period of a few hours after PGF2α release that a CL can be palpated and observed at ultrasonography but plasma progesterone concentration would be <1 ng mL^{-1} exposure, the CL may be observed at ultrasonography but CL regression is evident atpalpation per rectum (i.e., hard CL and uterine tone). Detection of follicles was based on ultrasonography, uterine tonicity using per rectum palpation and uterine edema using bothultrasonography and per rectum palpation. Per rectum palpation and

Table 1: Criteria for determination of the stage of the estrous cycle or presence of ovarian cysts or anestrus based on ultrasonography and per rectum palpation of the genital tract

	Clinical findings	
Stages	Ovaries	Uterus
Diestrus	Functional CL, follicle >10 mm	Slight tonus
Metestrus	Corpus hemorrhagicum, follicle <10 mm	Edema and moderate tonus
Proestrus/ estrus	Follicle _ 18 mm, regressing CL	High tonus
Ovarian cysts	Multiple follicles _ 18 mm, absence of CL	Flaccid
Anestrus	Follicle <18 mm	Flaccid

ultrasonography of thegenital tract have been previously used for the diagnosis of ovarian cysts. In the present study, the diagnosis of ovarian cysts was based on three clinical findings:

- Multiple follicles approximately 18 mm in diameter
- Absence of a CL
- Lack of uterine tonicity

Collectively, these studies indicate that a large follicle (e.g., 25 mm) cannot be used as the sole criterion for cysts since multiple follicles reach ovulatory size indicating enough gonadotropin to support follicular development but these follicles fail to ovulate due to lack of GnRH/LH surge. The absence of uterine tonicity is the most important clinical finding that can be used to make a diagnosis on a single examination since it indicates that regression of the CL has occurred, the cow has failed to ovulate and the follicles are continuing to grow in the absence of high progesterone concentrations.

Ovarian ultrasonography: Transrectal ultrasonography was conducted in groups of cows using a portable scanner (Easi-scan, BCF Technology Ltd., Livingston, UK) fitted with a 6 MHz linear-array transducer to determine the ovarian structures at the Journal of Dairy Science Vol. 90 No. 12, 2007 1st GnRH injection of the Resynch protocols and 7 days later at the PGF2α injection. Diameter of the follicle and CL was estimated using on-screen background gridlines comprising squares with 10 mm sides as described previously. Ovulation was defined as the presence of a follicle on the day of 1st ultrasound examination and presence of a CL in the same location of the ovary 7 days later at the 2nd ultrasound examination. Because of missing data at the 1st GnRH injection or at PGF2α injections.

Statistical analysis: Baseline comparisons for parity (1, 2, 3+), season (August to September and October toDecember), DIM (quartiles), inseminator (A-F), stages of the estrous cycle (diestrus, metestrus and proestrus), ovarian cysts or anestrus were carried out to establish comparability among groups using a Chi-square test (PROC FREQ, SAS system).

Pregnancy rates on days 27, 45 and 90 and pregnancy losses between days 27 and 45, 45 and 90 for both groups were compared using Chi-square. In addition, pregnancy rates on days 27, 45 and 90 were compared using a multivariate analysis to adjust for differences in baseline data and to evaluate the interactions between groups and stages of the estrouscycle or presence of ovarian cysts or anestrus on pregnancy rate.

The model included group, stage (i.e., stages of the estrous cycle or presence of ovarian cysts or anestrus), parity, season, DIM, inseminator and their interactions and they remained in the model at p<0.15 using the backward elimination method of the logistic regression procedure in PROC GENMOD, SAS. Treatment differences with p<0.05 were considered significant whereas tendencies were considered when p>0.05.

RESULTS AND DISCUSSION

When ultrasound is used to diagnose pregnancies at post-AI (day 30), resynchronization ovulation program with a GnRH injection in cows of unknown pregnancy status and non-pregnant diagnosis seems to be contraindicated because the resulting P/AI are not improved. For herds in which pregnancy diagnosis is made at post-AI (day 30) either by trans rectal ultrasound or palpation initiating the resynchronization program requires GnRH to improve the resulting fixed-time P/AI. Changing the timing of the 2nd GnRH to 56 h after PGF2α and inseminating 16 h later (72 h post-PGF2α) clearly improved conception rate and fixed time P/AI compared with 2nd GnRH to 48 h after PGF2α. Coupling a non-pregnancy diagnosis with a management decision to quickly reinseminate cows may improve reproductive efficiency by decreasing the interval between AI services. Delaying the 1st injection of GnRH for Resynch until 30 days after initial TAI yielded more pregnancies for all treated cows. Although, it is desirable to reinseminate cows as soon as possible after pregnancy status is known and adoption of ultrasound provides an opportunity to conduct such a diagnosis, programming cows to receive the 1st GnRH of Resynch 30 days after the initial TAI. Cows in anestrus were not considered in the statistical analysis due to the low number of cows in this category. Therefore, pregnancy rates were evaluated for 3 groups that 1st group (n = 50) 18 pregnant and 32 non-pregnant, pregnancy rate in this group 36% and cows in the group 2 (n = 50) 11 pregnant and 39 non-pregnant, pregnancy rate in this group 22% and cows in the group 3 (n = 50), 16 pregnant and 34 non-pregnant, pregnancy rate in this group 32% and there was significant difference in pregnancy rate between group 1 and 2, p = 0.0044 (p<0.05), pregnancy rate between group 2 and 3 was significant too, p = 0.0446 (p<0.05) and there was no significant difference between pregnancy rate in group 1 and 3, p = 0.5202 (p>0.05).

Differences were detected between treatment groups for pregnancy rates and resynchronization reduced DIM at TAI and total days open after the initial TAI (Table 2-5).

Table 2: Pregnancy rate difference between groups 1 and 2

		Pregnancy diagnosis			
Groups	Rate	Positive	Negative	Total	p-value
PG	Prevalence	18	32	50	0.0044
	Percentage	36	64	100	
Ovsynch 48 h	Prevalence	11	39	50	
	Percentage	22	78	100	

Table 3: Pregnancy rate difference between groups 1 and 3

		Pregnancy diagnosis			
Groups	Rate	Positive	Negative	Total	p-value
PG	Prevalence	18	32	50	0.5202
	Percentage	36	64	100	
Ovsynch 56 h	Prevalence	16	34	50	
	Percentage	32	68	100	

Table 4: Pregnancy rate difference between group 2 and 3

		Pregnancy diagnosis			
Groups	Rate	Positive	Negative	Total	p-value
Ovsynch 48 h	Prevalence	11	39	50	0.3419
	Percentage	22	78	100	
Ovsynch 56 h	Prevalence	16	34	50	
	Percentage	32	68	100	

Table 5: Pregnancy rate and p-value in 3 groups

	Groups		
Variables	PG (%)	Ovsynch 48 h (%)	Ovsynch 56 h (%)
Pregnancy rate	36 (18)	22 (11)	32 (16)
	Between 1 and 2	Between 2 and 3	Between 1 and 3
p-value	0.0044	0.0446	0.5202

Because estrus expression and detection are reduced in high producing lactating dairy cows (Britt, 1995) resynchronization of estrus needs to be combined with resychronization of ovulation and TAI.

Considering that the stage of the estrous cycle affects the efficacy of TAI protocols (Fissore *et al*., 1986; Lares *et al*., 2002) different strategies for resynchronization and TAI have been considered. Pregnancy rates were similar in cows with or without a CL at per rectum examination of the genital tract and then assigned to different protocols that combined estrus detection and TAI (Bartolome *et al*., 2005b). The use of ultrasonography permitted the initiation of GnRH for resynchronization to a non-pregnancy diagnosis at 30 days after previous AI (Fricke *et al*., 2003; Nebel *et al*., 2008).

The prevalence of anestrous cows after the end of the voluntary waiting period is an important factor affecting the reproductive efficiency of dairy herds (Pieterse *et al*., 1990; Szenci *et al*., 1998). Resynchronization programs are frequently used for the reproductive management of dairy cattle to improve the reinsemination rate of non-pregnant cows and to expedite the interval from non-pregnancy to conception (Caraviello *et al*., 2006). Previous research has focused on the fertility of dairy cows after a single resynchronization treatment (Chebel *et al*., 2003; Colazo *et al*., 2003) without considering the time to pregnancy when cows are subjected to the same treatment over time. Fricke *et al*. (2003) attempted to evaluate pregnancy risk when cows were subjected to resynchronization with GnRH either before or at the diagnosis of non-pregnancy but treatments were altered because of a reduced resynchronized pregnancy rate when GnRH was given on day 23 after AI. The current study evaluated different time (48 and 56 h) of treatments to resynchronize return to estrus and ovulation in dairy cows.

Analyses of concentrations of progesterone in plasma on day 14, 21 and 24 were valuable to evaluate the possible effect of treatments on the presence of CL and luteolysis because increases (Chebel *et al*., 2003) or decreases (Chenault *et al*., 2003) in the conception rate to the P/AI have been observed in previous studies when used resynchronization of return to estrus.

Anestrous cows had a marked reduction in reproductive performance and these effects were independent of treatment. Reduced detection of estrus and decreased.

Pregnancy rates were observed in cows classified as anestrous compared with cyclic. Such effects in anestrous cows having delayed cyclicity, reduced detection of estrus (Curran *et al*., 1986; Laing *et al*., 1976) and reduced pregnancy rates (Curran *et al*., 1986; Laing *et al*., 1976) have been observed. When enrolled in a TAI program, anestrous cows do not benefit from resynchronization with PGF2α which reduces the benefits of resynchronization on pregnancy (Peters and Pursley, 2002). Anestrous cows alsohave reduced synchronized ovulation which compromises pregnancy likely by reducing fertilization or by influencing embryo quality when fertilization is delayed and oocyte quality is compromised. Furthermore, anestrous cows are more likely to experience short luteal cycles after 1st ovulation which is linked to a decreased number of progesterone receptors and an increased number of oxytocin receptors in the endometrial cells resulting from inadequate exposure to progesterone which might trigger premature release of PGF2α and luteolysis.

Conception rates on 48 and 56 h resynchronization methods indicate that treatment of lactating dairy cows of unknown pregnancy status with GnRH did affect the established pregnancy and are in accordance with previous studies in which GnRH (Chebel *et al*., 2003; Fricke *et al*., 2003) were administered to lactating dairy cows of unknown pregnancy status.

CONCLUSION

The results of this study show that when cows were diagnosed non-pregnant by ultrasonography at 30 days post-AI to a previous service, resynchronization of ovulation and TAI with Ovsynch 48 and 56 h protocols resulted in similar pregnancy rates. However, the Ovsynch 56 h protocol increased pregnancy rate for cows at the time of initiation of the treatment protocol.

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