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Effect of pre-treatment follicular size on reproductive response of dairy heifers after PG2α induced estrus and GnRH administration on the day of artificial insemination

S. A. Yotov* and A.S. Atanasov

Department of Obstetrics, Reproduction and Reproductive Disorders, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

Abstract

The present study was designed to investigate the influence of pre-treatment follicular size on the reproductive response of dairy heifers after PGF2á injection and GnRH administration on the day of artificial insemination (AI). After transrectal ultrasound examination, fifty-two dairy heifers bearing corpus luteum (CL) were selected and divided into three groups: Group I (n=19) with small follicles (2-5 mm); Group II (n=18) with medium follicles (6-9 mm) and Group III (n=15) with dominant follicle (10-12 mm). Each group was administered 500 mg PGF2α immediately after ultrasound examination (Day 0) and 100 μg GnRH at the time of AI, performed 80 hours after PGF2α injection. A second ultrasound examination was done early morning on Day 3 (72 h) after prostaglandin treatment and presence of a pre-ovulatory follicle and clinical estrus signs were recorded. All inseminated heifers were monitored to a clinical estrus expression by daily observation and new AI was made in the cases of spontaneous estrus detection. Ultrasound pregnancy diagnosis was done on Day 30 and on Day 70 after the first AI. Heifers with medium and dominant follicles showed a better ($P<0.05$) reproductive response in terms of uterine tone, relaxation of cervix, and pregnancy rate after induced estrus. These findings indicated that pre-treatment follicular size has influence on prostaglandin induced estrus characteristics and pregnancy rate following GnRH administration on the day of AI in dairy heifers.

Keywords: Dairy heifers; Follicular Size; PG2α; GnRH

Introduction

Hormonal manipulation of estrous cycle and artificial insemination are used successfully for improvement of reproductive efficiency in dairy herds (Odde, 1990; Whisnant *et al.*, 1999; Mapletoft *et al.*, 2003; Sartori *et al.*, 2008; Alnimer *et al.*, 2011). Effective management of reproduction requires optimization of insemination process and preparing of replacement heifers on time (Rajala-Schultz *et al.*, 2000). However,

*Corresponding Author: stanrad@abv.bg
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most of the heifers are inseminated after spontaneous estrus (Pursley *et al.*, 1995). One of the mean reasons for less than expected reproductive improvement from AI is inadequate estrus detection and the consequent failure to inseminate within the optimal period (Erven and Arbaugh, 1987; Caraviello *et al.*, 2006). In organized dairy rearing systems, this problem can be partly overcome by estrus synchronization programs coupled with timed AI.

One widespread method for estrus synchronization includes single injection of luteolytic dose PGF2α in animals bearing CL (Stellflug *et al*,. 1975; Sirois and Fortune 1988). Application of PGF2á during luteal phase of the estrous cycle leads to estrus expression with or without ovulation from day 3 to day 5 days after treatment (Larson *et al.*, 1996; Martinez *et al.*, 2000). Another schedule is a double administration of prostaglandin (PGF2α) 11 or 14 days apart (Macmillan and Henderson, 1984; Morbeck *et al.*, 1991). Regardless of this, the interval between PGF2α injection and ovulation is rater variable which leads to insemination out of the optimal period and low conception rate (Archbald *et al.*, 1992; Xu and Burton, 1999). The problem is explained with different stage of follicles at the start of the treatment (Thatcher *et al.*, 1996; Larson *et al.*, 1996; Lamb *et al.*, 2006).

A combination of gonadotropin-releasing hormone (GnRH) and PGF2α has been used in many estrus synchronization protocols to improve conception and pregnancy rate in dairy heifers (Moreira *et al.*, 2000; Rivera *et al.*, 2004; Tasdemir *et al.*, 2011). Ovsynch program have been developed for timed artificial insemination (TAI) without estrus detection. The treatment includes GnRH injection on Day 0, PGF2α on Day 7, and second GnRH on Day 9, followed by timed AI (TAI) 0 to 24 h later (Pursley *et al.*, 1995; Schmitt *et al.*, 1996). Alternative version of the previous program is Co-Synch protocol, but AI has to be made on the day of the second GnRH administration (Geary and Whittier, 1997). The success of both the schedules also depends on follicular development (Moreira *et al.*, 2000). Application of pre-synchronization before the onset of GnRH-PG (Sterry *et al.*, 2007; Stevenson, 2011) could be effective in heifers, but rises significant treatment cost (Whisnant *et al.*, 1999).

Most of the synchronization programs including GnRH and PGF2α have been applied successfully in lactating dairy cows but low results were registered in heifers (Savio *et al.*, 1988; Pursley *et al.*, 1997, 1998; Demüral *et al.*, 2006). According to Rivera *et al.* (2005) dairy heifers could express estrus close to the PGF2α injection, thereby causing asynchrony at TAI. Furthermore greater percentage of the heifers has three wave follicular growth patterns (Wolfenson *et al.*, 2004; Šcihtar *et al.*, 2010) that could be a reason for reduced effect of estrus synchronization.

In dairy cows various authors (Dolezel *et al.*, 2002; Bartolome *et al.*, 2005;

Herlihy *et al.*, 2012; Gumen *et al.*, 2012) applied ultrasound determination of ovarian status before treatment. Similar investigations have been made in heifers by Moreira *et al.* (2000) and Demüral *et al*. (2006) before Ovsynch and Cosynch protocols. The attempts for optimization of TAI programs in dairy heifers continue to be developed (Krueger and Heuwieser, 2011; Tasdemir *et al.*, 2011), but the results are still debatable. According to Macmillan (2010), nutrition and environmental factors like heat stress have profound effects on the physiology and metabolism of the high producing dairy animals and different estrus synchronization protocols have not been able to overcome the consequences of lowered fertility.

The current study describes the influence of follicular size on the reproductive response of dairy heifers after PGF2α injection and GnRH administration on the day of artificial insemination.

Materials and methods

Forty dairy heifers from Bulgarian Brown cattle breed and twenty crossbreds (Bulgarian Brown x Red Holstein) without estrus behavior during the monthly observation were presented to a transrectal ultrasonography. The examination was made by ultrasound scanner SonoScape A5 Vet (SonoScape Co. LTD, Shenzhen, China) and 7-12 MHz linear transducer and ovarian status was recorded. Fifty-two animals bearing corpus luteum in one of the ovaries were selected and divided into three groups: Group I (n=19) with small follicles (2-5 mm); Group II (n=18) with medium follicles (6-9 mm) and Group III (n=15) with dominant follicle (10-12 mm). All heifers were clinically healthy with 380-400 kg body weight, aged 18-24 months and body condition scores 3.5-4 by the scale of Edmonson *et al.* (1989). The experiment was carried out in the hot season (July-September).

The treatment included administration of 500 mg PGF2á (2 ml PGF Veyx forte, Veyx-Pharma GmbH, Schwarzenborn, Germany) immediately after ultrasound examination (Day 0) followed 80 hours latter by 100 µg GnRH (Depherelin, Veyx-Pharma GmbH, Schwarzenborn, Germany) at time of AI. A second ultrasound examination was carried out early morning on Day 3 (72 h) after prostaglandin treatment and the presence of a pre-ovulatory follicle (diameter > 12 mm) was recorded. The clinical estrus signs (good uterine tone; ease of cervical passage and cervical mucous discharge) were determined by the methods of Stevenson *et al.* (1983) and Loeffler *et al.* (1999). Artificial insemination was done with frozen semen from one high producing bull. Post AI, all inseminated heifers were monitored for clinical estrus expression by daily observations - three times per day. In a case of spontaneous estrus detection each animal was inseminated artificially during the standing phase. Ultrasound pregnancy diagnosis was done on Day 30 and on Day 70 after the first AI.

Reproductive response endpoints included detection of a pre-ovulatory follicle and presence of the clinical estrus signs at the day of first AI, pregnancy rate after induced and spontaneous estrus, pregnancy rate at day 30 and pregnancy losses from day 30 to 70, total value of each reproductive parameter and overall pregnancy rate for all experimental period.

Statistical analysis was performed with the Stat-Soft 1984–2000 Inc. statistical software (Copyright ©1990–1995 Microsoft Corporation) by a non-parametric analysis for comparison of proportions, using Student's t-criterion. Differences were considered significant at the P<0.05 level.

Results

The results from the study are presented in Table 1. On the day of first AI pre-ovulatory follicle was observed in 68.4%, 83.3% and 66.7% of animals in groups with small, medium and dominant follicles, respectively. Total value of 70.1% was calculated for all 52 heifers.

A good uterine tone was determined in 68.4% of cases from groups I. This value differed significant (P<0.05) than values in group II (94.4%) and group III (100%). The cases of ease of cervical passage (100%) in group II and III were much more than these (68.4%) in group I (P<0.05). The percentage of animals in group I (47.4%) exhibiting cervical mucus discharge was similar to that in group II (55.6%). Insignificantly increase (73.3%) was noted in the heifers with a dominant follicle. Total values of uterine tone, ease of cervical passage, and cervical mucus discharge were 86.5%, 88.5% and 57.5%, respectively.

The pregnancy rate after induced estrus 42.1% in group I was significant lower (P<0.05) than pregnancy rate 72.2% in groups II and 66.7% in group III, but statistically difference between the last two groups was not determined.

The percentage of non-conceived animals after AI expressing spontaneous estrus latter was almost identical, 21.1%, 16.7% and 20% in first, second and third group, respectively. Total pregnancy rate was 59.6%.

Pregnancy losses from day 30 to 70 were the lowest in group II (11.1%) and similar to that (13.3%) in group III, but differed (P<0.05) than received values (36.8%) in group I. Moreover, three heifers with small follicles at the start of treatment were presented with ovarian cysts during the ultrasound on Day 30 after AI.

The pregnancy diagnosis after the second ultrasonography was negative in 21.2% of animal. All positive diagnoses from Day 30 were confirmed. Overall, pregnancy rate after induced and spontaneous estrus 88.9% (15/18) in heifers with medium follicles was similar to pregnancy rate 86.7% (13/15) in animals with dominant follicle. The percentage (63.3%) in group with small follicles was less than the above two values in P<0.05.

Table1: Reproductive parameters in dairy heifers treated with PG2α and GnRH on the day of artificial insemination.

Groups	Pre-ovulatory follicle at the day of first AI		Clinical estrus signs						Pregnancy rate				Non-pregnant heifers up to day 70th after treatment	
			Good uterine tone		Ease of cervical passage		Cervical mucus discharge		After induced estrus		After spontaneous estrus			
n	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Group I small follicles (n=19)	13/19	68.4	13/19	68.4[a]	13/19	68.4 [a]	9/19	47.4	8/19	42.1 [a]	4/19	21.1	7/19	36.8[a]
Group II medium follicles (n=18)	15/18	83.3	17/18	94.4[b]	18/18	100 [b]	10/18	55.6	13/18	72.2 [b]	3/18	16.7	2/18	11.1[b]
Group III dominant follicle (n=15)	10/15	66.7	15/15	100[b]	15/15	100 [b]	11/15	73.3	10/15	66.7 [b]	3/15	20	2/8	13.3[ab]
Total n=52	38/52	70.1	45/52	86.5	46/52	88.5	30/52	57.7	31/52	59.6	10/52	19.2	11/52	21.2

Values within a column bearing different superscripts differ significantly at $P<0.05$.

Discussion

The present study shows that follicular development at the beginning of the PGF2α treatment influence reproductive response of dairy heifers. Regardless of corpus luteum presence follicular size is an important factor to development of a pre-ovulatory follicle after this treatment. This confirms data of Rivera *et al.* (2004) in dairy heifers and Dole *et al.* (2002) in dairy cows to differences in follicular growth depending on their size at day of prostaglandin injection.

Our results indicate that pre-ovulatory follicle is available in 70.1% of animals at 72^{th} hour after prostaglandin treatment with the lowest values (66.7%) in the heifers with a dominant follicle at the beginning. It is in agreement with study of Leitman *et al.* (2008) who report to mean interval from PG to ovulation of 77.2±4.32 hours. The absence of pre-ovulatory follicles in animals from group I could be due to incomplete luteal regression after PG2α administration. Incomplete luteal regression and slow follicular growth in dairy heifers after PG treatment is stated by Rivera and Frike (2002) and Rantala *et al.* (2009). However, some heifers without pre-ovulatory follicle in group II (n=2) and III (n=3) showed good uterine tone, ease of cervical passage without a visible corpus luteum into the ovaries that could correspond with earlier ovulation. According to Dol *et al.* (2002) large follicles presented at the time of prostaglandin treatment ovulated very early.

The current data shows that animals with a dominant follicle at the start of the treatment have predominance in cervical mucous discharge. We suggest that longer exposition of the animals from group three of estrogens from dominant follicle corresponds with more vigorously discharge from the cervix. Herlihy *et al.* (2012) anounced that increased preovulatory follicle size and greater circulating concentrations of E_2 is due to a longer period of preovulatory follicle growth. Nevertheless we consider that it is not prerequisite to high pregnancy rate using GnRH for induction of ovulation, which is confirmed from obtained pregnancy rate results.

The pregnancy rate after induced estrus in heifers with small follicles was less than obtained pregnancy rate, when medium and large follicles are present in the ovaries. It could be caused from insufficient maturation of a follicle at time of GnRH administration and absence of ovulation. The time from PGF2α administration to ovulation was dependent on the maturity and size of the most emergent dominant follicle, because a small dominant follicle takes longer to grow into an ovulatory follicle (Kastelic and Ginther, 1991). Moreira *et al.* (2000) investigated effect of day of the estrous cycle at the initiation of a timed artificial insemination in dairy heifers, using Ovsynch protocol. They demonstrated that frequency of ovulation after the second injection of GnRHa was 80% for groups with mean follicular diameter 4.6-10 mm and 100% for groups with mean follicular diameter 11-12.5 mm detected at Day 0 of the synchronization period.

Total pregnancy rate (59.6%) is in disagreement with the results (35%) of Pursley *et al.* (1997) after Ovsynch but coincide with the reported 59.6% by Tasdemir *et al.* (2011), who used two PGF2α injections 14 days apart followed by GnRH at 72h after 2nd PGF2á at the time of TAI. A similar pregnancy rate was reported by Krueger and Heuwieser (2011) after PG2α treatment and AI during the standing estrus. The present data also shows that non-pregnant animals after the first AI could be inseminated after spontaneous estrus and most of them became pregnant. It is confirmed by insignificant differences between the groups in parameter pregnancy rate after spontaneous estrus. The registration of significant ($P<0.05$) higher percentage of non-pregnant animals in group I for all experimental period could be explained with cysts diagnosis in three of cases.

Overall pregnancy rate is an additional indicator for better reproductive response in presence of medium or dominant follicle into the ovaries than small one at the start of PGF2α treatment and GnRH administration at time of AI.

In our opinion, selection of dairy heifer according to ovarian follicular status before PG-GnRH treatment and TAI could improve synchronization and pregnancy rate. The protocol used in this study shows acceptable reproductive effects, especially in animals bearing corpus luteum and medium and dominant follicles at the beginning of the treatment. It may be used as a schedule with TAI and optimization of reproductive performance in dairy heifers.

In conclusion, follicular size has influence on the prostaglandin induced estrus in dairy heifers. In animals with corpus luteum and medium and dominant follicles, PGF2α administration followed 80 hours latter by GnRH and AI has effective reproductive response. It could be useful to reproductive performance improvement.

References

Alnimer, M.A., Alfataftah, A.A and Ababneh, M.M. 2011. A comparison of fertility with a Cosynch protocol versus a modified Ovsynch protocol which included estradiol in lactating dairy cows during the summer season in Jordan. *Anim Reprod,* **8:**32-39.

Archbald, L.F., Tran, T., Massey, R. and Klapstein, E. 1992. Conception rates in dairy cows after timed-insemination and simultaneous treatment with gonadotropin releasing hormone and/or prostaglandin F2 alpha. *Theriogenology,* **37:**723-731.

Bartolome, J.A., Sozzi, A., McHale, A., Swift, K., Kelbert, D., Archbald, L.F. and Thatcher, W.W. 2005. Resynchronization of ovulation and timed insemination in lactating dairy cows: III Administration of GnRH 23 days post AI and ultrasonography for nonpregnancy diagnosis on day 30. *Theriogenology,* **63:**1643-1658.

Caraviello, D.Z., Wiegel, K.A., Fricke, P.M., Wiltbank, M.C., Florent, M.J., Cook, N.B., Nordlund, K.V., Zwald, N.R.and Rawson, C.L. 2006. Survey of management practices on reproductive performance of dairy cattle on large US commercial farms. *J Dairy Sci,* **89:**4723-4735.

Demüral, O., Mustafa, U.N., Abay, M., Bekyrek, T. and Ozturk, A. 2006. The Effectiveness of Cosynch Protocol in Dairy Heifers and Multiparous Cows. *Turk J Vet Anim Sci,* **30:**213-217**.**

Dolezel, R., Cech, S., Zajic, J. and Havlicek, V. 2002. Oestrus synchronization by PGF2á and GnRH in intervals according to stage of follicular development at time of initial treatment in cows. *Acta Vet Brno,* **71:**101-108.

Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T. and Webster, G. 1989. A body condition scoring chart for Holstein dairy cows. *J Dairy Sci,* **72:**68-78.

Erven, B.L. and Arbaugh, D. 1987. Artiûcial insemination on U.S.dairy farms. Report of a study conducted in cooperation with the National Association of Animal Breeders. NAAB, Columbia, MO.

Geary, T.W. and Whittier, J.C. 1997. Modification of the Ovsynch estrous synchronization protocol for use in beef cows. *J Anim Sci,* **75 (Suppl. 1):**236 (Abstr.).

Gumen, A., Keskin, A., Yilmazbas-Mecitoglua, G., Karakaya, E., Alkan, A., Okut, H. and Wiltbank, M.C. 2012. Effect of presynchronization strategy before Ovsynch on fertility at ûrst service in lactating dairy cows. *Theriogenology,* **78:**1830-1838.

Herlihy, M.M., Crowe, M.A., Diskin, M.G. and Butler, S.T. 2012. Effects of synchronization treatments on ovarian follicular dynamics, corpus luteum growth, and circulating steroid hormone concentrations in lactating dairy cows. *J. Dairy Sci.,* **95:**743-754.

Kastelic, J.P. and Ginther, O.J., 1991. Factors affecting the origin of the ovulatory follicle in heifers with induced luteolysis. *Anim Reprod Sci,* **26:**13-24.

Krueger, H. and Heuwieser, W. 2011. Effect of CIDR on 4-day-service-rate, pregnancy rate and vaginal irritation in dairy heifers. *Tierärztl Prax.,* **39 (G):**277-280.

Lamb, G.C., Larson, J.E., Geary, T.W., Stevenson, J.S., Johnson, S.K., Day, M.L., Ansotegui, R.P., Kesler, D.J., DeJarnette, J.M. and Landblom, D.G. 2006. Synchronization of estrus and artificial insemination in replacement beef heifers using gonadotropin-releasing hormone, prostaglandin F2á, and progesterone. *J Anim Sci,* **84:**3000-3009.

Larson, R.L., Corah, L.R. and Peters, C.W. 1996. Synchronization of estrus in yerling beef heifers with the melengestrol acetate/prostaglandin F2á system: efficiency of timed insemination 72 hours after prostaglandin treatment. *Therio-genology,* **45:**851-863.

Leitman, N.R., Busch, D.C., Bader, J.F., Mallory, D.A., Wilson, D.J., Lucy, M.C., Ellersieck, M. R., Smith, M.F. and Patterson, D.J. 2008. Comparison of protocols to synchronize estrus and ovulation in estrous-cycling and prepubertal beef heifers. *J Anim Sci,* **86:**1808-1818.

Loeffler, S.H., De Vries, M.J., Schukken, Y.H., De Zeeuw, A.C., De Graaf, F.M. and Brand, A. 1999. Use of AI technician scores for body condition, uterine tone and uterine discharge in a model with disease and milk production parameters to predict pregnancy risk at first AI in Holstein dairy cows. *Theriogenology,* **51:**1267-1284.

Macmillan, K.L. 2010. Recent advances in the synchronization of estrus and ovulation in dairy cows. *J Reprod Dev,* **56 (Suppl):**42-47.

Macmillan, K.L. and Henderson, H.V. 1984. Analyses of the variation in the interval from an injection of prostaglandin F2 alpha to oestrous as a method of studying patterns of follicle development during dioestrous in dairy cows. *Anim Reprod Sci,* **6:**245-254.

Mapletoft, R.J., Martínez, M.F., Colazo, M.G. and Kastelic, J.P. 2003. The use of controlled internal drug release devices for the regulation of bovine reproduction. *J Anim Sci,* **81:**E28-E36.

Martinez, M.F., Adams, G.P., Kastelic, J.P., Bergfelt, D. and Mapletoft, R.J. 2000. Induction of follicular wave emergence for estrus synchronization and artiûcial insemination in heifers. *Theriogenology,* **54:**757-769.

Morbeck, D.E., Tyler, H.D. and Britt, J.H. 1991. Duration of estrous cycles subsequent to two injections of prostaglandin F2á at a 14 day interval in non-lactating Holstein cows. *J Dairy Sci,* **74:**2342-2346.

Moreira, F., De la Sota, R.L., Diaz, T. and Thatcher, W.W. 2000. Effect of day of estrous cycle at the initiation of a timed artiûcial insemination protocol on reproductive responses in dairy heifers. *J Anim Sci,* **78:**1568-1576.

Odde, K.G. 1990. A review of synchronization of estrus in postpartum cattle. *J Anim Sci,* **68:** 817-830.

Pursley, J.R., Mee, M.O. and Wiltbank, M.C. 1995. Synchronization of ovulation in dairy cows using PGF 2á and GnRH. *Theriogenology,* **44:**915-923.

Pursley, J.R., Wiltbank, M.C., Stevenson, J.S., Ottobre, J.S., Garverick, H.A. and Anderson, L.L. 1997. Pregnancy rates per artiûcial insemination for cows and heifers inseminated at a synchro-nized ovulation or synchronized estrus. *J Dairy Sci,* **80:**295-300.

Pursley, R.J., Silcox, R.W. and Wiltbank, M.C. 1998. Effect of time of artiûcial insemination on pregnancy rates, calving rates, pregnancy loss and gender ratio after synchronization of ovulation in lactating dairy cows. *J Dairy Sci,* **81:**2139-2144.

Rajala-Schultz, P.J., Gröhn, Y.T. and Allore, H.G. 2000. Optimizing breeding decisions for Finnish dairy herds. *Acta Vet Scand,* **41:**199-212.

Rantala, M.H., Katila, T. and Taponen, J. 2009. Effect of time interval between prostaglandin F2 alpha and GnRH treatments on occurrence of short estrous cycles in cyclic dairy heifers and cows. *Theriogenology,* **71:**930-938.

Rivera, H. and Fricke, P.M. 2002. Synchronization of estrus in dairy heifers using GnRH, PGF 2á and ECP. *J Dairy Sci,* **85 (Suppl. 1):**267. (Abstr.).

Rivera, H., Lopez, H. and Fricke, P.M. 2004. Fertility of holstein dairy heifers after synchronization of ovulation and timed AI or AI after removed tail chalk. *J Dairy Sci,* **87:**2051-2061.

Rivera, H., Lopez, H. and Fricke, P.M. 2005. Use of intravaginal progesterone releasing inserts in a synchronization protocol before timed AI and for synchronizing return to estrus in Holstein heifers. *J Dairy Sci,* **88:**957-958.

Sartori, R., Baruselli, P.S., Souza, A.H., Cunha, A.P. and Wiltbank, M.C. 2008. Recent advances in ovulation synchronization and superovulation in dairy cattle. *Anim Reprod,* **6:**194. (Abstracts).

Savio, J.D., Keenan, L., Boland, M.P. and Roche, J.F. 1988. Pattern of growth of dominant follicles during the oestrous cycle of heifers. *J Reprod Fertil,* **83:**663-671.

Schmitt, E.J.P., Diaz, T., Drost, M. and

Thatcher, W.W. 1996. Use of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin for timed insemination in cattle. *J Anim Sci,* **74:**1084-1091.

Sichtar, J., Tolman, R., Rajmon, R., Klabanová, P., Berka, P. and Volek, J. 2010. A comparison of the follicular dynamics in heifers of the Czech Fleckvieh and Holstein breeds. *Czech J Anim Sci,* **55:**234-242.

Sirois, J. and Fortune, J.E. 1988. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. *Biology Reprod,* **39:**308-317.

Stellflug, J.N., Louis, T.M., Hafs, H.D. and Seguin, B.E. 1975. Luteolysis, estrous and ovulation, and blood prostaglandin F after intramuscular administration of 15, 30 or 60 mg prostaglandin F2alpha. *Prostaglandins,* **9:**609-615.

Sterry, R.A., Jardon, P.W. and Fricke, P.M. 2007. Effect of timing of Cosynch on fertility of lactating Holstein cows after ûrst postpartum and resynch timed-AI services. *Theriogenology,* **67:**1211-1216.

Stevenson, J.S. 2011. Alternative programs to presynchronize estrous cycles in dairy cattle before a timed artificial insemination program. *J Dairy Sci,* **94:**205-217.

Stevenson, J.S., Schemidt and Call, E.P. 1983. Estrous intensity and conception rate in Holsteins. *J Dairy Sci,* **66:**275-280.

Taºdemir, U., Yilmazbaº-Mecitoðlu, G., Keskin, A., Karakaya, E., Çelik, Y., Güzeloðlu, A. and Gümen, A. 2011. Conception rate following timed artificial insemination protocols in dairy heifers synchronised by PGF2á and GnRH. *Ankara Üniv Vet Fak Derg,* **58:**135-139.

Thatcher, W.W., De la Sota, R.R., Schmitt, E.J-P., Diaz, T.C., Badinga, L., Simmen, F.A., Staples, C.R. and Drost, M. 1996. Control and management of ovarian follicles in cattle to opti-mize fertility. *Reprod Fertil Dev,* **8:**203-217.

Whisnant, C.S., Washburn, S.P. and Farin, P.W. 1999. Current concepts in synchronization of estrus and ovulation of dairy cows. *Proceedings of the American Society of Animal Science*: 1-8.

Wolfenson, D., Inbar, G., Roth, Z., Kaim, M., Bloch, A. and Braw-Tal, R. 2004. Follicular dynamics and concentrations of steroids and gonadotropins in lactating cows and nulliparous heifers. *Theriogenology,* **62:**1042-1055.

Xu, Z.Z. and Burton, L.G. 1999. Reproductive performance of dairy heifers after estrus synchronization and ûxed-time artiûcial insemination. *J Dairy Sci,* **82:**910-917.