ACTA VET. BRNO 2022, 91: 125–131; https://doi.org/10.2754/avb202291020125

The effect of supplementation of follicle-stimulating hormone (FSH) into the Ovsynch protocol to increase the pregnancy rate in cyclic lactating dairy cows

Gulnaz Yilmazbas-Mecitoglu[1], Abdulkadir Keskin[1], Ebru Karakaya-Bilen[2], Umut Taşdemir[3], Ahmet Gumen[1]

[1]University of Bursa Uludag, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, Bursa, Turkey
[2]University of Siirt, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, Siirt, Turkey
[3]Aksaray University, Faculty of Veterinary Medicine, Department of Artificial Insemination, Aksaray, Turkey

Received September 23, 2021
Accepted February 1, 2022

Abstract

The aim of this study was to determine the effects of the follicle stimulating hormone (FSH) supplemented in the Ovsynch protocol on pregnancy per timed artificial insemination (P/TAI) in cyclic dairy cows. All cows (n = 383) included in the study received the Ovsynch protocol. The cows in FSH3 (n = 92), FSH4 (n = 88), and FSH3&4 (n = 91) were administered 20 mg FSH on day 3, or day 4, or both on days 3 and 4 of the protocol, respectively, whereas the control group (n = 112) did not receive any FSH treatment. The mean follicle number at TAI and ovulation number after TAI were similar among the FSH groups. However, the mean follicular size was smaller (~14.9 mm, $P = 0.02$) in FSH4 compared to the other groups (~15.9 mm). Pregnancy/TAI of the FSH4 group on day 31 (59.1%) and day 62 (53.4%) was higher than those of other groups on day 31 (45.5% in control, 45.7% in FSH3, and 46.2% in FSH3&4) and day 62 (42.9% in control, 43.5% in FSH3, and 40.7% in FSH3&4), but the difference was non-significant. Likewise, FSH4 (62.7%) had more ($P = 0.05$) P/TAI than the other groups (48.6% in control, 47.7% in FSH3, and 49.4% in FSH3&4) in synchronized cows. The cows which responded to the first gonadotropin-releasing hormone (GnRH) of Ovsynch in the FSH4 group (64.8%) had higher ($P < 0.02$) P/AI rate than the responding control cows (44.6%). We concluded that application of FSH on day 4 in cyclic cows which were responsive to the Ovsynch protocol can yield higher pregnancy rates, but more studies are needed to better demonstrate this potential effect.

Cattle, FSH, synchronization, fertility

Reproductive efficiency is one of the most important components of a profitable dairy system. The physiological and environmental stresses of high milk production, inadequate nutrient intake, low body condition, and intensive management systems impair reproductive performance in dairy cows (Butler 2000; Thatcher et al. 2000). The most withholding physiological factor on fertility in lactating dairy cows is low steroid hormone concentrations in the pre- and post-insemination periods, since elevated feed intake in high-producing dairy cows is associated with higher hepatic blood flow resulting in higher catabolism of steroid hormones in the liver (Wiltbank et al. 2006). The most notable effect of lower oestradiol concentrations on reproductive performance is the decrease in oestrus duration in these high producing dairy cows. Thus, the Ovsynch protocol, a timed artificial insemination (TAI) protocol decreasing the reliance on oestrous detection on dairy farms, has become a popular and successful tool by increasing the service rate without labour investment for oestrus detection (Pursley et al. 1997). However, the fertility achieved with the Ovsynch protocol is suboptimal since the protocol stimulates premature ovulation of the follicle, preventing oestradiol concentrations to reach further levels (Souza et al. 2007). Premature ovulation of the follicle also results in smaller corpus luteum (CL) and lower progesterone concentrations

Address for correspondence:
Gulnaz Yilmazbas-Mecitoglu
Department of Obstetrics and Gynaecology
Faculty of Veterinary Medicine
University of Bursa Uludag
Gorukle Campus, 16059, Bursa, Turkey

Phone: +90 224 294 08 27
E-mail: gulnazy@uludag.edu.tr
http://actavet.vfu.cz/

in the post-AI period (Vasconcelos et al. 1999), which is a critical risk factor for early embryonic loss (Wiltbank et al. 2000).

Stimulation of two follicles instead of one can be beneficial for overcoming the above mentioned negative factors which are effective both in the pre- and post-insemination periods of the Ovsynch protocol. Double ovulation leads to increased pregnancy rates because of either an increase in fertility at AI for cows having multiple follicles or a reduction in early pregnancy loss for cows having multiple CL (Fricke and Wiltbank 1999). The prominent hormonal factor in the aetiology of double ovulation is reported as the differences in follicle stimulating hormone (FSH) concentrations which occur for a very brief period near the time of the deviation phase of follicular wave (Wiltbank et al. 2000). Also, it is known that the FSH surge is responsible not only for the emergence of a new follicular wave but also the selection of the dominant follicle in the new wave. The selection of the dominant follicle coincides with the FSH concentrations lowering during the first three days of the wave, with the lowest value observed 4 days after the emergence (Lopez et al. 2005; Adams et al. 2008).

Thus, our objectives in this study were; 1) to determine the optimum time of FSH that would increase the number of the dominant follicle at TAI and the number of CL following TAI, and 2) to determine whether treatment of FSH would increase pregnancy rates in cyclic lactating dairy cows receiving the Ovsynch protocol.

Materials and Methods

Animals

The experiment was conducted on 383 cyclic, lactating Holstein cows in a commercial dairy herd with approximately 1,000 lactating dairy cows in the South Marmara region, Bursa, Turkey. The cows were housed in free-stall facilities and grouped according to their milk production. All cows were milked 3 × a day and the milk production of the herd was 9,880 ± 69.7 kg (for 305 days) per cow. Average milk production for each cow was recorded for seven days before and after insemination. The cows received a mixed ration balanced to meet the minimal nutritional requirements according to the National Research Council (NRC 2001), and had free access to water. Daily milk yield, reproductive health, and management records for each cow were collected from Alpro 2000 Herd Management System (DeLaval, Tumba, Sweden). Just before the initiation of the study, the body condition scores (BCS) of all cows were recorded. The scoring was based on a scoring system (1 = thin to 5 = obese) proposed by Ferguson et al. (1994). The experimental procedures were approved by the Lalahan Livestock Central Research Institute Animal Care Committee (No: 2009/27).

Study design

The cows were treated with the Ovsynch protocol (GnRH—7 d—$PGF_{2\alpha}$—56 h—GnRH—16/18 h—TAI). The first GnRH (Receptal®, buserelin acetate, 10 µg, i.m., Intervet, Istanbul, Turkey) of Ovsynch was administered on the day the cows were examined ultrasonographically and determined as cyclic (having at least one CL at any of the ovaries). Seven days after GnRH, $PGF_{2\alpha}$ (Estrumate®, cloprostenol, 500 µg, i.m., CEVA-DIF, Istanbul, Turkey) was administered. A second GnRH treatment was administered 56 h after $PGF_{2\alpha}$ and all cows were inseminated at a fixed time 16 to 18 h after the final GnRH treatment using frozen-thawed semen.

All the cows that underwent the Ovsynch protocol were randomly assigned to one of the four experimental groups at the initiation of the protocol. The cows in FSH3 (n = 92), FSH4 (n = 88), and FSH3&4 (n = 91) groups were administered FSH (Folltropin®, 20 mg., i.m., Bioniche, Inverin, Ireland) on day 3, or day 4, or both on days 3 and 4, respectively, after the first GnRH of Ovsynch, whereas the control group (n = 112) did not receive any FSH treatment and served as control.

Ultrasonographic examinations were performed by Honda HS 2000 ultrasonograph equipped with a 7.5 MHz transducer (Honda, Tokyo, Japan) at the beginning of the study (at the time of the first GnRH) to determine the cyclicity and maximal follicle sizes, at the time of $PGF_{2\alpha}$ administration to determine CL on the ovary, and at the time of TAI to determine the number and size of the follicles. Diameters of the potentially ovulatory follicles were obtained by averaging perpendicular measurements of the cross-sectional diameter for each follicle. Ultrasonographic examinations were also done 7 days after TAI to determine the number of CL to confirm ovulation(s) of the dominant follicle(s). Ovulatory response after the GnRH treatments was determined by the disappearance of the dominant follicle and presence of a new CL on the ovary 7 days after the first GnRH and after TAI. No dominant follicle on the ovary with/without a newly developed CL at the time of TAI indicated early ovulation, whereas no CL on the ovary 7 d after TAI was considered as no ovulation.

Pregnancy was diagnosed with ultrasonography after the visualization of a fluid-filled uterine horn with embryonic vesicles on day 31 and the presence of a foetus on day 62 following TAI.

Table 1. Ovarian responses to the Ovsynch protocol, follicle stimulating hormone (FSH) treatments, and pregnancy rates in cyclic dairy cows.

Item		CON	FSH3	FSH4	FSH3&4	P value
Number of follicle at TAI		1.45 ± 0.06[b]	1.55 ± 0.07[ab]	1.61 ± 0.10[ab]	1.77 ± 0.09[a]	0.03
Number of ovulation on d 7 post-TAI		1.15 ± 0.038	1.10 ± 0.032	1.15 ± 0.043	1.20 ± 0.047	0.15
Follicle size at TAI, mm		15.95 ± 0.24[a]	15.85 ± 0.26[a]	14.88 ± 0.27[b]	15.85 ±0.27[a]	0.02
Ovulation to first GnRH		58.0% (65/112)	56.5% (52/92)	61.4% (54/88)	53.8% (49/91)	0.78
Synchronizatin Rate						
(Ovulation to second GnRH)		93.8% (105/112)	95.7% (88/92)	94.3% (83/88)	92.3% (84/91)	0.81
Cows with multiple CL on d 7 post-TAI		14.3% (15/105)	10.2% (9/88)	13.3% (11/83)	19.0% (16/84)	0.10
Pregnancy/TAI, % (n/total)	At 31 d	45.5% (51/112)	45.7% (42/92)	59.1% (52/88)	46.2% (42/91)	0.18
	At 62 d	42.9% (48/112)	43.5% (40/92)	53.4% (47/88)	40.7% (37/91)	0.32
	Pregnancy loss	5.9% (3/51)	4.7% (2/42)	9.6% (5/52)	11.9% (5/42)	0.31
	One CL*	48.9% (44/90)	48.1% (38/79)	56.9% (41/72)	48.5% (33/68)	0.20
	≥ 2 CL*	46.6% (7/15)[a]	44.4% (4/9)[a]	100% (11/11)[b]	56.2 % (9/16)[a]	0.01

FSH3, FSH4, FSH3&4 - cyclic cows treated with FSH treatment on day 3 or on day 4 or both on day 3 and 4 of the Ovsynch protocol, respectively; CON - cyclic cows that received the Ovsynch protocol but no FSH treatment; TAI - timed artificial insemination; CL - corpus luteum
Values followed by the same superscript within the row do not differ significantly

The ovulation rate was calculated by dividing the number of cows that responded to the second GnRH to the total number of cows in the group. The pregnancy rate was calculated by dividing the number of cows diagnosed pregnant at day 31 to the number of cows inseminated. The embryonic loss was calculated as the number of cows diagnosed non-pregnant at 62 d post TAI, divided by the number of cows diagnosed pregnant at 31 days post-TAI.

Statistical analysis

Statistical analyses were conducted by using SAS (Version 9.2; SAS Institute, 2010). Data were evaluated using PROC LOGISTIC, PROC GLM, and PROC FREQ in SAS. Statistical models were constructed that included the lactation number, treatment groups, milk production, BCS, days in milk (DIM), follicle size and number at the time of TAI, and the number of follicles resulting with ovulation post-TAI. Data were coded as 1 (yes) or 0 (no) for the ovulatory response to the first and second GnRH. Ovulation number on day 7 post-TAI was coded as 1 (1 CL) or 2 (more than one CL). Pregnancy on days 31 and 62 after TAI was coded as 0 (non-pregnant) or 1 (pregnant).

The PROC GLM procedure was performed to compare the milk production, DIM, BCS, lactation number, follicle size and follicle number at the time of TAI, and the number of follicles that resulted with ovulation post-TAI among the groups. Chi-square analysis using the PROC FREQ procedure was used: to compare the ovulatory response to first and second GnRH, and P/TAI on day 31 and day 62; and to compare multiple CL on days 31 and 62 in pregnant cows. PROC LOGISTIC was used to analyze the effect of treatment, milk production, DIM, BCS, follicle size at the time of TAI, response to the first GnRH of Ovsynch, and lactation number on pregnancy rate on days 31 and 62. A forward stepwise selection procedure was utilized to construct the final model, and differences with $P < 0.05$ were considered significant.

Results

General results

No significant differences were detected in DIM, BCS, and milk production, except lactation number of cows ($P = 0.02$; 1.9 ± 0.12 in control, 2.5 ± 0.14 in FSH3, 2.2 ± 0.15 in FSH4, and 2.2 ± 0.13 in FSH3&4 groups). When the ovulatory response to GnRH administration of Ovsynch was evaluated, the response to the first GnRH of Ovsynch did not differ among groups (58.0%, 65/112 in control; 56.5%, 52/92 in FSH3; 61.4%, 54/88 in FSH4; and 53.8%, 49/91 in FSH3&4). In addition, the synchronization rate (ovulation to the second GnRH of Ovsynch) was also similar among the groups and approximately 93% of the cows were synchronized (Table 1).

Pregnancy rates

Total pregnancy/TAI was detected as 48.8% (187/383) in all cows regardless of the treatment group. When P/TAI was evaluated in cows responsive to the first GnRH of the protocol, the responsive cows had numerically greater P/TAI (51.4%, 113/220) than the non-responsive cows (44.8%, 73/163) ($P < 0.20$). Pregnancy/TAI at day 31 was similar among the treatment groups and it was detected as 45.5% in control, 45.7% in FSH3, 59.1% in FSH4, and 46.2% in FSH3&4 (Table 1). In addition, P/TAI on day 62 was also similar among the groups (Table 1). No significant difference was observed among the groups regarding the embryonic loss (5.9%, 3/51 in control; 4.7%, 2/42 in FSH3; 9.6%, 5/52 in FSH4; and 11.9%, 5/42 in FSH3&4).

The effect of FSH treatment on the mean number of the follicle, ovulation, and CL

The follicle number of cows at the time of TAI in the FSH3&4 group (1.77 ± 0.09) was significantly greater (P=0.03) than in the control group (1.45 ± 0.06; Table 1). The mean number of the ovulated dominant follicle after TAI did not differ among the groups (~ 1.15). However, the mean follicle diameter was detected lower (P = 0.02) in the FSH4 group (14.88 ± 0.27 mm) compared with the other groups (~ 15.88 mm, Table 1). In addition, the percentage of cows with multiple CL post-TAI did not differ among the groups (Table 1).

The effect of FSH treatment on pregnancy rates

When the effect of FSH treatments on pregnancy rates in responsive cows to first GnRH was evaluated, the FSH4 group (64.8%) had greater ($P < 0.02$) P/TAI on day 31 than responsive cows in the control group (44.6%) and tended to be greater ($P < 0.06$) than responsive cows in the other groups (50.0% in FSH3 and 46.9% in FSH3&4, Table 2). Similarly, P/TAI in cows responsive to the first GnRH in the FSH4 group (67.3%) was higher ($P < 0.05$) than responsive cows in the other groups (46.8% in control, 51.0% in FSH3, and 46.9% in FSH3&4) in synchronized cows (Table 2). However, there was no difference in pregnancy rates among the groups regarding the non-responsive cows to first GnRH (Table 2).

Table 2. Pregnancy/TAI according to the response to the Ovsynch protocol among the groups.

	All cows		Synchronized cows	
	GnRH (+)	GnRH (−)	GnRH (+)	GnRH (−)
CON	44.6% (29/65)[a]	46.8% (22/47)	46.8% (29/62)[a]	51.2% (22/43)
FSH3	50.0% (26/52)[ab]	40.0% (16/40)	51.0% (26/51)[ab]	43.2% (16/37)
FSH4	64.8% (35/54)[b]	50.0% (17/34)	67.3% (35/52)[b]	54.8% (17/31)
FSH3&4	46.9% (23/49)[ab]	45.2% (19/42)	51.1% (23/45)[ab]	48.7% (19/39)

FSH3, FSH4, FSH3&4 - cyclic cows treated with FSH treatment on day 3 or on day 4 or both on day 3 and 4 of the Ovsynch protocol, respectively; CON - cyclic cows that received the Ovsynch protocol but no FSH treatment; TAI - timed artificial insemination

[a,b] - $P < 0.05$

In synchronized cows, when the P/TAI on day 31 was evaluated, the FSH4 group (62.7%, 52/83) had a greater ($P < 0.05$) P/TAI than the other groups (48.6%, 51/105 in control; 47.7%, 42/88 in FSH3; and 50.0%; 42/84 in FSH3&4) regardless of response to first GnRH.

Pregnancy/TAI in the cows which ovulated more than two follicles following TAI (60.8%, 31/51) was numerically greater with 10 percentage unit increment than cows which ovulated one follicle (50.5%, 156/309). Furthermore, cows that ovulated more than two follicles after TAI in the FSH4 group had more ($P < 0.01$) P/TAI on day 31 than the other groups (Table 1).

Discussion

To the authors' knowledge, the present study is the first one to evaluate the effect of FSH supplementation into the Ovsynch protocol. Previously, other researchers investigated supplementation of equine chorionic gonadotropin (eCG), which has both FSH and luteinizing hormone (LH) activity, at different times of TAI protocols (Souza et al. 2009; Kenyon et al. 2012).

The ovulatory response to the first and second GnRH of the Ovsynch protocol is important to obtain a successful synchronization outcome. Increased ovulation to first GnRH administration leads to a greater synchronization rate after $PGF_{2\alpha}$ and the highest ovulation rates after the second GnRH administration (Vasconcelos et al. 1999). Synchronization is essential to the coordination of a new functional dominant follicle at the time of $PGF_{2\alpha}$ and subsequent ovulation following the final GnRH of Ovsynch, thus higher pregnancy rates. The ovulatory responses to the first and second GnRH of Ovsynch in the present study were detected as 57% to first and 93% to second GnRH of the protocol which are similar to the rates previously reported as around 45–75% to first (Vasconcelos et al. 1999; Galvão and Santos 2010; Keskin et al. 2010; Yilmazbas-Mecitoglu et al. 2013) and 70–90% to second GnRH administrations (Vasconcelos et al. 1999; Navanukraw et al. 2004; Yilmazbas-Mecitoglu et al. 2013). The pregnancy rate obtained in the present study was 48.8% regardless of the treatment group, in accordance with the previously reported rates (35–50%) (Pursley et al. 1997; Keskin et al. 2010; Yilmazbas-Mecitoglu et al. 2013). Although the pregnancy rates were similar among the cows treated or non-treated with FSH, our results indicated that the pregnancy rates in cows treated with FSH on day 4 in the Ovsynch protocol were numerically higher (by at least 10 percentage unit) compared to the other FSH and control groups.

A more precise assessment of the effect of FSH treatments on pregnancy rates has become possible in cows that responded to the first GnRH and started a new follicular wave. The pregnancy rate in the FSH4 group in our study was significantly better in cows that had an ovulatory response to the first GnRH.

According to our hypothesis, increased pregnancy rates after addition of FSH to the Ovsynch protocol would be related to the multiple follicles at TAI or multiple CLs post-TAI period or both, improving the local environmetal factors effective on the reproductive organs. However, since the mean numbers of follicles at TAI (~1.5) or ovulated follicles following TAI (~1.15) were similar among the groups, the higher pregnancy rate in the FSH4 group seemed to be due to a smaller follicle size at the time of TAI (14.9 mm in FSH4 group vs ~15.9 mm in other groups). Follicular sizes in our study were within the ranges (13.5–17.5 mm) associated with higher pregnancy rates in a previous study (Keskin et al. 2016). However, ovulation from smaller and younger follicles has been suggested to result in higher pregnancy rates in other studies (Wiltbank et al. 2000; Lynch et al. 2010) and this can be the situation in our FSH4 group.

No increase in the pregnancy rate was observed in cows either with one or two follicles at TAI or one or two CLs post-TAI, except the FSH4 group (Table 1). It might be pointed out that the follicle size, which is suggestive of the oocyte quality, is more effective on pregnancy rate rather than having multiple follicles or multiple CLs. Previous studies indicated that high embryo survival is associated with better oocyte quality in smaller follicles (Lynch et al. 2010) and that the fertility reduces in larger follicles due to aging oocyte (Mihm et al. 1996; Revah and Butler 1996).

It seems more ideal to administer FSH on day 4 of the Ovsynch protocol because it corresponds to the very first day of the selection phase which occurs at least two days after the possible emergence of the new follicular wave (Wiltbank et al. 2000). The emergence of the wave is estimated to occur two days following the first GnRH of the protocol in responsive cows (Pursley et al. 1997; Sellars et al. 2006).

In the present study, the increase in the pregnancy rate of cows in FSH4 group could not be detected in the FSH3&4 group, although animals in the latter group received the hormone on day 4 in addition to day 3. This situation may be due to the possible oocyte quality decreasing effect of early or repeated doses of FSH resulting from early or overstimulation of the dominant follicle on the selection phase. The selection process of the potential dominant follicle is optimized by FSH and FSH-dependent growth factors (Mihm and Austin 2002) via regulating LH receptors (LHR) of the potential dominant follicles (Sirard et al. 2007). The first FSH administration on day 3 in the FSH3&4 group coinciding with the day before the probable selection phase of GnRH-induced follicular wave may have resulted in early stimulation of the selection process. On the other hand, the repeated doses of exogenous FSH given on days 3 and 4 of the protocol, together with the endogenous physiological FSH, may have resulted in overstimulation.

Thus, the early administration and/or repeated doses of exogenous FSH in the FSH3&4 group somehow had a disrupting effect on the regulation of LHR of the future dominant follicle(s) and/or oocyte quality. It was reported that the superovulation protocols enable a more physiological pattern of LHR expressions and result in better oocyte maturation or superovulatory response (Simões et al. 2012).

As a conclusion, although the FSH treatment was initiated in the present study as early as on day 3 of the protocol in order not to miss the selection phase of the GnRH-induced follicular wave, the FSH treatment on day 4 seems more effective and beneficial for cyclic lactating dairy cows with better pregnancy rates. Thus, we suggest that the modification of the Ovsynch protocol with the supplementation of FSH on day 4 and beyond in the Ovsynch protocol has a high potential to achieve better pregnancy rates, however, further studies are needed to better demonstrate this effect.

Acknowledgement

We greatly acknowledge Assoc. Prof. Dr. I. Taci Cangul for language revision and proofreading.

References

Adams GP, Jaiswal R, Singh J, Malhi P 2008: Progress in understanding ovarian follicular dynamics in cattle. Theriogenology **69**: 72-80

Butler WR 2000: Nutritional interactions with reproductive performance in dairy cattle. Anim Reprod Sci **60-61**: 449-457

Ferguson JD, Galligan DT, Thomsen N 1994: Principal descriptors of body condition score in Holstein cows. J Dairy Sci **77**: 2695-2703

Fricke PM, Wiltbank MC 1999: Effect of milk production on the incidence of double ovulation in dairy cows. Theriogenology **52**: 1133-1143

Galvão KN, Santos JEP 2010: Factors affecting synchronization and conception rate after the Ovsynch protocol in lactating Holstein cows. Reprod Domest Anim **45**: 439-446

Kenyon AG, Lopes G Jr, Mendonça LG, Lima JR, Bruno RG, Denicol AC, Chebel RC 2012: Ovarian responses and embryo survival in recipient lactating Holstein cows treated with equine chorionic gonadotropin. Theriogenology **77**: 400-411

Keskin A, Mecitoğlu G, Bilen E; Güner B; Orman A, Okut H, Gumen A 2016: The effect of ovulatory follicle size at the time of insemination onpregnancy rate in lactating dairy cows. Turk J Veter Anim Sci **40**: 68-74

Keskin A, Yilmazbas-Mecitoglu G, Gumen A, Karakaya E, Darici R, Okut H 2010: Effect of hCG vs. GnRH at the beginning of the Ovsynch on first ovulation and conception rates in cyclic lactating dairy cows. Theriogenology **74**: 602-607

Lopez H, Sartori R, Wiltbank MC 2005: Reproductive hormones and follicular growth during development of one or multiple dominant follicles. Biol Reprod **72**: 788-795

Lynch CO, Kenny DA, Childs S, Diskin MG 2010: The relationship between ovulatory endocrine and follicular activity on corpus luteum size, function, and subsequent embryo survival. Theriogenology **73**: 90-198

Mihm M, Austin EJ 2002: The final stages of dominant follicle selection in cattle. Domest Anim Endocrinol **23**: 155-166

Mihm M, Diskin MG, Roche JF 1996: Regulation of follicle wave growth in cattle. Reprod Dom Anim **31**: 531-538

Navanukraw C, Redmer DA, Reynolds LP, Kirsch JD, Grazul-Bilska AT, Fricke PM 2004: A modified presynchronization protocol improves fertility to timed artificial insemination in lactating dairy cows. J Dairy Sci **87**: 1551-1557

Pursley JR, Kosorok MR, Wiltbank MC 1997: Reproductive management of lactating dairy cows using synchronization of ovulation J Dairy Sci **80**: 301-306

Revah I, Butler WR 1996: Prolonged dominance of follicles and reduced viability of bovine oocytes. J Reprod Fertil **106**: 39-47

Sellars CB, Dalton JC, Manzo R, Day J, Ahmadzadeh A 2006: Time and incidence of ovulation and conception rates after incorporating estradiol cypionate into a timed artificial insemination protocol. J Dairy Sci **89**: 620-626.

Simões RAL, Satrapa RA, Rosa FS, Piagentini M, Castilho ACS, Erena RL, Trinca LA, Nogueira MFG, Burantini J Jr, Barros CM 2012: Ovulation rate and its relationship with follicle diameter and gene expression of the LH receptor (LHR) in Nelore cows. Theriogenology **77**:139-147

Sirard MA, Desrosier S, Assidi M 2007: In vivo and in vitro effects of FSH on oocyte maturation and developmental competence. Theriogenology **68**: 71-76

Souza AH, Gumen A, Silva EPB, Cunha AP, Guenther JN, Peto CM 2007: Supplementation with estradiol-17b before the last GnRH of the Ovsynch protocol in lactating dairy cows. J Dairy Sci **90**: 4623-4634

Souza AH, Viechneski S, Lima FA, Silva FF, Araújo R, Bó GA, Wiltbank MC, Baruselli PS 2009: Effects of equine chorionic gonadotropin and type of ovulatory stimulus in timed-AI protocol on reproductive responses in dairy cow. Theriogenology **72**: 10-21

Thatcher WW, Moreira F, Santos JEP 2000: Strategies to improve reproductive management of dairy cows. Adv Dairy Technol **12**:177-193

Vasconcelos JLM, Silcox RW, Rosa GJ, Pursley JR, Wiltbank MC 1999: Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology **52**: 1067-1078

Wiltbank MC, Lopez H, Sartori R, Sangsritavong S, Gumen A 2006: Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology **65**: 17-29

Wiltbank MC, Fricke PM, Sangritasvong S, Sartori R, Ginther OJ 2000: Mechanisms that prevent and produce double ovulations in dairy cattle. J Dairy Sci **83**: 2998-3007

Yilmazbas-Mecitoglu G, Karakaya E, Keskin A, Alkan A, Gumen A 2013: Reducing the duration between gonadotropin-releasing hormone (GnRH) and prostaglandin F2α treatment in the Ovsynch protocol to 6 days improved ovulation to second GnRH treatment, but inclined to reduce fertility. J Dairy Sci **96**: 3817-3824