Haryana Vet. (March, 2020) **59(SI),** 65-68 **Research Article**

COMPARISON OF PRE-OVULATORY FOLLICLE AND CORPUS LUTEUM SIZE FOLLOWING GnRH AND hCG BASED SYNCHRONIZATION PROTOCOLS IN REPEAT BREEDER COWS

AMIT KASHYAP, MADHUMEET SINGH, AKSHAY SHARMA* and PRAVESH KUMAR
Department of Veterinary Gynecology and Obstetrics, College of Veterinary and Animal Sciences,
Himachal Pradesh Agricultural University, Palampur–176062, India

Received: 04.07.2018; Accepted: 05.10.2018

ABSTRACT

The objective of present study was to evaluate the difference between pre-ovulatory follicle size and subsequent corpus luteum in repeat breeder cows following synchronization with Ovsynch (GnRH based) and modified Ovsynch (hCG based) protocols. The mean diameter of pre-ovulatory follicle in the cows treated with GnRH (11.48±0.44 mm;n=7) was significantly lower ($p<0.05$) in comparison to hCG (14.31±1.18 mm; n=7) treated cows.The mean diameter of corpus luteum on day 6 post AI in the cows treated with GnRH (16.11±0.45 mm; n=7) was significantly higher ($p<0.05$) than hCG (13.37±1.66 mm; n=7) treated cows.The difference between mean pre-ovulatory follicular diameter in pregnant (11.54±0.50 mm; n=5) and non-pregnant (11.35±1.25 mm; n=2) cows was not statistically significant ($p>0.05$) but difference between mean corpus luteum diameter on day 6 post AI in pregnant (16.7±0.37 mm; n=5) and non-pregnant (14.65±0.15 mm; n=2) cows was statistically significant ($p<0.05$) in GnRH based synchronization group. However, in hCG based synchronization group, mean pre-ovulatory follicle and corpus luteum diameter in pregnant and non-pregnant cows varied non-significantly ($p>0.05$).

Keywords: Cows, GnRH, hCG, Repeat breeder,Synchronization protocols

Reproductive performance is one of the most important factors affecting dairy farm profitability and the development of national economy, as well as the living standard of rural and urban societies. There are many factors that affect reproductive performance, such as high milk production, negative energy balance, low detection rate for estrus, and increased embryonic loss in dairy cows. Therefore, size of the pre-ovulatory follicle could be an important factor for adequate luteal function and subsequent embryonic survival (Vasconcelos *et al.*, 2013). Several studies have also focused on the follicle size at the time of artificial insemination (AI) and the relations between follicle size and several aspects of the subsequent reproductive hormonal profile and fertility in lactating dairy cows (Lopes *et al.*, 2007; Kapse *et al.*, 2017). So the present study envisages the effectiveness of GnRH and hCG based synchronization protocols in formation of pre-ovulatory follicle and subsequent corpus luteum (CL).

MATERIALS AND METHODS

The present study was carried out in 14 repeat breeder cows of Instructional Livestock Farm Complex (ILFC) of CSK Himachal Pradesh KrishiVishvavidyalya, Palampur (32.6 ºN, 76.3 ºE, altitude 1290.8 m).In Group 1 (G1), estrus synchronization via Ovsynch protocol was initiated in 7 cows with the aim of initiation and development of the follicular wave on day 0 i.e., day 6 of estrous cycle and 10 µg Buserelin acetate was administered by intramuscular route. On day 7, i.e. day 13 of the estrous cycle, 500 µg Cloprostenol was administered intramuscularly as an exogenous source for initiation of the lysis of CL. On day 9, i.e. day 15 of the estrous cycle, 10 µg Buserelin acetate was administered with the aim of supporting the ovulation of tertiary follicle developed in the new wave and fixed timed artificial insemination (FTAI) was done on day 10, i.e. day 16 of estrous cycle and day 11, i.e. day 17 of estrous cycle. In Group 2 (G2), Modified Ovsynch protocol for synchronization was started in 7 cows on day 0 with the aim of initiation and development of the follicular wave and 3000 I.U. of hCG was administered by intramuscular route. On day 7, 500 µg Cloprostenol was administered intramuscularly as an exogenous source for initiation of the lysis of the corpus luteum. On day 9, 3000 I.U. of hCG was administered intramuscularly with the aim of supporting the ovulation of the tertiary follicle developed in the new wave and FTAI was done on day 10 and 11. The impact of treatment on follicular dynamics was evaluated by measuring the size of the largest dominant follicle and CL. Size of dominant follicle and CL was determined by taking mean of follicular and CL diameter at the widest point and perpendicular to the first measurement using internal calipers on the ultrasound machine (Z5 Vet, Mindray; 7.5 MHz).The data obtained were statistically analyzed using Student's t-test for parametric data as described with SAS (Statistical Analysis Software), SAS® 9.2 TS Level 2M2 for windows.

RESULTS AND DISCUSSION

The mean diameter of pre-ovulatory follicle in the cows treated with GnRH based Ovsynch protocol was smaller (11.48±0.44 mm; $p<0.05$) in comparison to hCG based Modified Ovsynch (14.31±1.18 mm) treated cows (Table 1). Contrarily, Diaz *et al.* (1998) reported formation

*Corresponding author: akshays482@gmail.com

Table 1

Mean (±SE) diameter of pre-ovulatory follicle at 9 day of protocol and subsequent corpus luteum 6 days post AI

Sr. No.	Protocol	Diameter (Mean±SE) of pre-ovulatory Follicle (mm)	Diameter (Mean±SE) of subsequent Corpus luteum on day 6 post AI (mm)
1	Ovsynch (GnRH based) (n=7)	11.48±0.44[a]	16.11±0.45[a]
2	Modified Ovsynch (hCG based) (n=7)	14.31±1.18[b]	13.37±1.66[b]

a,b Values with different superscripts within the same column differ significantly (p<0.05)

of a smaller dominant follicle after hCG administration. The mean diameter of CL on day 6 post AI in the cows treated with GnRH based Ovsynch protocol was significantly larger (16.11±0.45 mm; p<0.05) in comparison to hCG based modified Ovsynch protocol (13.37±1.66 mm) treated cows. The follicle size alone is not sufficient for predicting the further development of a corpus luteum. Rather, both follicle size and estradiol concentration in the follicular fluid seems to be very important for the further development of a pre-ovulatory follicle into a healthy, actively secreting CL (Vernunft *et al.*, 2013). In our study, although the pre-ovulatory follicle size in hCG treated animals was larger (14.31±1.18 mm) in comparison to GnRH treated cows (11.48±0.44 mm), but low number of LH receptors on the follicle may contribute towards formation of dysfunctional CL (Roche and Diskin, 2000). In our study, hCG protocol resulted in ovulation of a follicle after the second injection of hCG and formation of small sized CL. It is known that luteal development is a continuation of follicular maturation; consequently, therefore, decreased gonadotropin stimulation or estradiol production of the follicle, may impair subsequent luteal function (Dickinson *et al.*, 2016).

In present study, difference between mean pre-ovulatory follicular diameter in pregnant (11.54±0.50 mm; n=5) and non-pregnant (11.35±1.25 mm; n=2) cows was not significant (p>0.05) in GnRH based synchronization protocol but the difference between mean corpus luteum diameter on day 6 post AI in pregnant (16.7±0.37 mm; n=5) and non-pregnant (14.65±0.15 mm; n=2) cows was statistically significant (p<0.05) in GnRH group. In hCG based synchronization group, mean pre-ovulatory follicular diameter in pregnant (21.2 mm; n=1) and non-pregnant (13.16±0.35, n=6) cows varied but the difference was not significant (p>0.05). Similarly, the difference between mean CL diameter in pregnant (20.00 mm; n=1) and non-pregnant (12.27±0.43 mm; n=6) cows varied widely but here also the difference was not significant (p>0.05) as shown in Table 2. In GnRH based group, mean pre-ovulatory follicle size was smaller as compared to hCG based groups but conception was higher in GnRH based group which may be due to the fact that GnRH resulted in ovulation of wide range of follicles whereas hCG has its ability to act on large size follicles only (Nogueira *et al.*, 2007). In support to our findings, Lynch *et al.* (2010) reported that smaller follicles resulted in higher CR. This may be due to the fact that larger follicles led to lower conception due to aged oocyte (Revah *et al.*, 1996). Additionally, smaller follicles were also found to be related to high embryo survival (Lynch *et al.*, 2010). When ovulation was induced with GnRH in cows, positive associations among ovulatory follicle size, circulating concentrations of pre-ovulatory estradiol, fertilization rate, embryo quality, circulating concentrations of progesterone during the postovulatory period, and pregnancy rate have been reported (Atkins *et al.*, 2013). Some studies have shown that larger ovulatory follicles at the time of AI increase CR (Keskin *et al.*, 2010; Sa Filho *et al.*, 2010). Studies have indicated that CR was higher following ovulation of approximately 16 mm dominant follicle (Perry *et al.*, 2005). Induced ovulation of small pre-ovulatory follicles, in cows that have not expressed estrus, may negatively impact acquisition of oocyte competence. While meiotic competence is mostly complete by the time follicle reaches 3 mm, inadequate cytoplasmic or molecular maturation could compromise oocyte competence in small pre-ovulatory follicles at GnRH-induced ovulation. Cows induced to ovulate small dominant follicles (<11.3 mm) experienced lower CR and higher incidence of late embryonic mortality than did those induced to ovulate large (>11.3 mm) dominant follicles (Dickinson *et al.*, 2016). These studies indicated that larger follicles were associated with higher estradiol levels which improve sperm and oocyte transportation in the reproductive tract and uterine environment for early embryonic development (Hawk, 1983).

In addition, the larger CL, which forms following ovulation from larger follicles, is capable of producing more progesterone. High progesterone level is an important factor in early embryonic development in lactating dairy cows (Lopes *et al.*, 2007; Wiltbank *et al.*, 2011). However, Perry *et al.* (2005) reported that follicle size alone did not influence subsequent concentrations of progesterone when cows spontaneously ovulated. Intra-follicular concentrations of estradiol may play a role in

Table 2

Relationship between follicular size at insemination and subsequent corpus luteum on day 6 post AI in pregnant and non-pregnant cows in different treatment groups

Sr. No.	Protocol		Diameter (Mean±SE) of pre-ovulatory Follicle (mm)	Diameter (Mean±SE) of corpus luteum on day 6 post AI (mm)
1	Ovsynch (GnRH based)	Pregnant (n=5)	11.54±0.50	16.70±0.37[a]
		Non-pregnant (n=2)	11.35±1.25	14.65±0.15[b]
2	Modified Ovsynch (hCG based)	Pregnant (n=1)	21.20	20.00
		Non-pregnant (n=6)	13.160.35	12.27±0.43

a,b Values with different superscripts within the same column differ significantly ($p<0.05$)

preparation of follicular cells for luteal formation and function (Reilly *et al.*, 1996). Therefore, cows that exhibited standing estrus with small follicular size may have attained concentrations of estradiol necessary to adequately prepare the follicular cells for luteinization regardless of follicular size (Perry *et al.*, 2005). Some researchers also reported that follicle size does not affect pregnancy in cows (Colazo *et al.*, 2009). Small size follicles lead to lower CR which may be due to the fact that LH receptor mRNA is undetectable in granulosa cells of small follicles whereas, LH receptor mRNA increased in accordance with follicular growth (Nogueira *et al.*, 2007). Lower CR in cows with small follicles could be a reflection of impotence of hCG for either ovulation induction or oocyte maturation. Moreover, this inadequate effect of hCG in cows with small follicles could be due to direct induction of ovulation without hypothalamo-hypophyseal axis leading to lower amount of LH. Therefore, small pre-ovulatory follicles could not be capable of ovulation following induction by hCG rather than induction by GnRH (Pancarci *et al.*, 2013). Perry *et al.* (2005) suggested that development of a normal CL depends on pre-ovulatory follicles having: (i) an adequate number of granulosa cells, (ii) an adequate number of luteinizing hormone receptors on granulosa and thecal cells, and (iii) granulosa cells capable of synthesizing adequate amounts of progesterone after luteinization. Therefore, lower CR after induced ovulation of smaller follicles might be the result of fewer granulosa cells present in the follicle before ovulation, resulting in fewer large luteal cells in the CL and subsequent lower progesterone concentrations. In another study, Wecker *et al.* (2012) noted that follicle and CL size were positively related, but no inference with conception was noted. However, in our study, positive relationship between follicle size and CL size with conception was present.

In nutshell, pre-ovulatory follicle size played a significant part in achieving conception; though failure of hCG based synchronization protocols in repeat breeder cows despite having larger follicular size may be due to incomplete transformation of granulosa cells to luteal cells which can be adjudged on the basis of small CL evidentpost ovulation. So a valid conclusion derived from the present study supports the use of GnRH based synchronization protocols in repeat breeder cows.

REFERENCES

Atkins, J.A., Smith, M.F., MacNeil, M.D., Jinks, E.M., Abreu, F.M., Alexander, L.J. and Geary, T.W. (2013). Pregnancy establishment and maintenance in cattle. *J. Anim. Sci.* **91**: 722-733.

Colazo, M.G., Gordon, M.B., Rajamahendran, R., Mapletoft, R.J. and Ambrose, D.J. (2009). Pregnancy rates to timed artificial insemination in dairy cows treated with gonadotrophin-releasing hormone or porcine luteinizing hormone. *Theriogenol.* **72**: 262-270.

Diaz, T., Schmitt, E.J., de la Sota, R.L., Thatcher, M.J. and Thatcher, W.W. (1998). Human chorionic gonadotropin-induced alterations in ovarian follicular dynamics during the estrous cycle of heifers. *J. Anim. Sci.* **76**: 1929-1936.

Dickinson, S.E., Geary, T.W., Monnig, J.M., Pohler, K.G., Green, J.A. and Smith, M.F. (2016). Effect of pre-ovulatory follicle maturity on pregnancy establishment in cattle: the role of oocyte competence and the maternal environment. *Anim. Reprod.* **13**(3): 209-216.

Hawk, H.W. (1983). Sperm survival and transport in the female reproductive tract. *J. Dairy Sci.* **66**: 2645-2660.

Kapse, S., Singh, M., Sharma, A. and Kumar, P. (2017). Dominant follicle diameter at insemination is related to positive pregnancy outcome in dairy cattle. *Indian J. Anim. Reprod.* **38**(1): 56-57.

Keskin, A., Mecitoglu, G.Y, Gumen, A., Karakaya, E., Darici, R. and 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.

Lopes, A.S., Butler, S.T., Gilbert, R.O. and Butler, W.R. (2007). Relationship of pre-ovulatory follicle size, estradiol concentrations and season to pregnancy outcome in dairy cows. *Anim. Reprod. Sci.* **99**: 34-43.

Lynch, C.O., Kenny, D.A., Childs, S. and Diskin, M.G. (2010). The relationship between ovulatory endocrine and follicular activity on corpus luteum size, function, and subsequent embryo survival. *Theriogenology.* **73**: 90-98.

Nogueira, M.F., Buratini, J.J., Price, C.A., Castilho, A.C., Pinto, M.G. and Barros, C.M. (2007). Expression of LH receptor Mrna splice variants in bovine granulosa cells: changes with follicle size and regulation by FSH in vitro. *Mol. Reprod. Dev.* **74**: 680-686.

Pancarci, S.M., Lehimcioglu, N.C., Ari, U.C., Gungor, O. and Akbulut, O. (2013). Efficacy of hCG and GnRH with respect to follicular size and presence of the corpus luteum in Co-synch protocol integrated with norgestomet in lactating cows. *Bull. Vet. Ins. Pulawy* **57**: 61-64.

Perry, G.A., Smith, M.F., Lucy, M.C., Green, J.A., Parks, T.E., MacNeil, M.D., Roberts, A.J. and Geary, T.W. (2005).

Relationship between follicle size at insemination and pregnancy status. *Pro. Natl. Acad. Sci.* **102**: 5268-5273.

Reilly, C.M., Cannady, W.E., Mahesh, V.B., Stopper, V.S., De Sevilla, L.M. and Mills, T.M. (1996). Concerted stimulation of rat granulosa cell deoxyribonucleic acid synthesis by sex steroids and follicle-stimulating hormone. *Biol. Reprod.* **54**:1336-1342.

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

Roche, J.F. and Diskin, M.D. (2000). Reproductive management of postpartum cows. *Anim. Reprod. Sci.* **60**: 703-712.

Sa Filho, M.F., Crespilho, A.M., Santos, J.E.P., Perry, G.A. and Baruselli, P.S. (2010). Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled Bos indicus cows. *Anim. Reprod. Sci.* **120**: 23-30.

Vasconcelos, J.L.M., Pereira, M.H.C., Meneghetti, M., Dias, C.C., SaFilho, O.G., Peres, R.F.G., Rodrigues, A.D.P. and Wiltbank, M.C. (2013). Relationships between growth of the pre-ovulatory follicle and gestation success in lactating dairy cows. *Anim. Reprod.* **10**: 206-214.

Vernunft, A., Weitzel, J.M. and Viergutz, T. (2013). Corpus luteum development and its morphology after aspiration of a pre-ovulatory follicle is related to size and steroid content of the follicle in dairy cows. *Vet. Med.* **58**: 221-229.

Wecker, F., Thedy, D.X., Gonsioroski, A.V. and Borges, J.B.S. (2012). Effect of administration of eCG or hCG 7 days after FTAI on the development of ovarian structures and pregnancy rates in beef cows. *Acta Vet. Scand.* **40**: 1072-1080.

Wiltbank, M.C., Sartori, R., Herlihy, M.M., Vasconcelos, J.L.M., Nascimento, A.B., Souza, A.H., Ayres, H., Cunha, A.P., Keskin, A. and Guenther, J.N. (2011). Managing the dominant follicle in lactating dairy cow. *Theriogenology.* **76**: 1568-1582.