



**Full Length Article**

## Optimization of the Estrus Synchronization Method and its Effect on Bulk Tank Milk Somatic Cell Count in Dairy Cattle Farming

Nevzat Saat<sup>1</sup>, Ali Risvanli<sup>2\*</sup>, Ibrahim Seker<sup>3</sup>, Erdal Kaygusuzoglu<sup>4</sup> and Abdurrahman Koseman<sup>5</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Balikesir, 10100, Balikesir, Turkey

<sup>2</sup>Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Firat, 23159, Elazig, Turkey

<sup>3</sup>Department of Zootechny, Faculty of Veterinary Medicine, University of Firat, 23159, Elazig, Turkey

<sup>4</sup>Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Bingol, 12100, Bingol, Turkey

<sup>5</sup>Department of Crop and Animal Production, Akcadag Vocational School, University of Inonu, 44100, Malatya, Turkey

\*For correspondence: arisvanli@firat.edu.tr

### Abstract

Effect of various estrus synchronization methods on bulk tank milk somatic cell count (BTMSSC) was investigated. One-hundred Simmental cows within a single dairy farm were randomized into four equal groups: no treatment (Group 1, control group); application of a progesterone-releasing intravaginal device (Group 2); administration of double-dose prostaglandin F<sub>2</sub> alpha (PGF<sub>2</sub>-alpha) with an 11-day interval (Group 3); and subjected to the Ovsynch protocol (Group 4). Bulk tank milk (BTM) samples were collected daily for one month and BTMSSCs calculated. Progesterone and estradiol concentrations were also measured at 3-day intervals. BTMSSC of the Ovsynch protocol group was not significantly different from that of the control group, suggestive of good udder health. Based on this finding, we propose that spontaneous estrus and the Ovsynch protocol are preferable methods for estrus synchronization than progesterone-releasing intravaginal device (PRID) and PGF<sub>2</sub>-alpha approaches. © 2017 Friends Science Publishers

**Keywords:** Estrus synchronization; Bulk tank milk; Somatic cell count; Dairy

### Introduction

The types and amounts of the cells within cow's milk can differ according to the physiological and pathological situation of the udder. The somatic cells within the milk are composed of epithelial cell residues in the mammary glands and channels, and the leukocytes and lymphocytes that pass into the milk from blood. Milk cell count is used in the diagnosis of subclinical mastitis (Bastan, 2013).

Various biochemical and microbiological tests are used in the diagnosis of subclinical mastitis. However, tests based on the somatic cell count (SCC) have recently gained importance. Although SCC is an important criterion, many factors (e.g., age and breed, lactation and sexual period, the dietary regimen of the animal, concomitant infections in the body and the bacterial species that lead to mastitis) can affect this count. Therefore, additional complementary tests are also recommended (Bastan *et al.*, 1997; Busato *et al.*, 2000).

Various approaches can be used to estimate SSC, including the California Mastitis Test (CMT), direct microscopic counting methods, the DNA filter method and Coulter counter and Fossomatic approaches. In normal milk, the SCC has been reported to be below  $2 \times 10^5$  cells·mL<sup>-1</sup>. This count was found to be between 3 and  $5 \times 10^5$  cells·mL<sup>-1</sup>

in CMT (+) animals, up to  $1 \times 10^6$  cells·mL<sup>-1</sup> in CMT (++) animals and greater than  $1 \times 10^6$  cells·mL<sup>-1</sup> in CMT (+++) animals. The samples to be examined for SCC in the diagnosis of subclinical mastitis may be obtained separately from each teat, from the combined milk or from the bulk tanks (Deveci *et al.*, 1994; Harmon, 1994; Furumura *et al.*, 1995).

The SCC is an important criterion for determining the quality of milk. Furthermore, individual or combined BTM follow-up SCC tests are important in the diagnosis of subclinical mastitis (Smith, 1996). However, many factors other than mastitis can also affect SCC. Thus, care should be taken when interpreting SCC data and diagnoses should be supported by other diagnostic tests. Nevertheless, high BTMSSC is considered an important problem worldwide. Currently, standard BTMSSC values differ between countries and between years within countries. However, with application of effective mastitis control programs, the number has decreased from  $7.5 \times 10^5$  cells·mL<sup>-1</sup> to as low as  $2 \times 10^5$  cells·mL<sup>-1</sup> in the United States (US) (Gillespie *et al.*, 2012; Barkema *et al.*, 2013).

Dairy cattle farmer aim for both high milk and breeding yield. However, simultaneously maintaining these two parameters is genetically impossible for the animals. The compromise of achieving high levels in one of these

traits, while maintaining optimal conditions for the second, is considered ideal. Recently, farmers have begun using synchronization protocols to increase milk yield, while preventing the fertility losses due to the increase in animal number. The condition of the farms should be considered when selecting these protocols and the problems encountered in that company. However, these protocols have been selected upon the increase in the breeding capacity rather than the milk quality and the mammary conditions (Aslan and Gumen, 2012; Semacan and Pancarci, 2012).

Various methods have been used to regulate the timing of estrus, ovulation and birth on cattle farms. Most of the estrus synchronization methods involve the synchronized luteolysis of corpus lutea or controlled termination of the diestrus period by forming an artificial diestrus period (Macmillan, 2010; Stevenson, 2016). Recently, the application of progesterone or PGF<sub>2</sub>-alpha and the Ovsynch method have come into prominence (Macmillan, 2010; Stevenson, 2016).

Moderate and large scale dairy cattle farming companies typically perform intensive breeding and employ various estrus synchronization protocols to regulate parturition timing. Such protocols typically aim to increase breeding yield and synchronizing parturition timings. However, udder health has generally been neglected in such approaches. A study conducted in 2014–2015 reported a mean BTMSCC  $<1.5 \times 10^5$  cells·mL<sup>-1</sup>. It was observed in this study that the BTMSCC values of the cattle had increased in the periods of synchronization protocols and the estrus timings. The aim of this project was to compare different estrus synchronization methods with regard to the effect on BTMSCC in the company and to determine the best protocol to minimize the increase in BTMSCC.

## Materials and Methods

The BTMSCC results of the Elazig farm (Turkey) were used, including 200 Simmental dairy cattle aged between 2 and 7 years. The mean BTMSCC of the herd was  $<150.000 \times 10^5$  cells·mL<sup>-1</sup> over the period of June 2014 to June 2015. The BTMSCC was seen to increase during periods of estrus synchronization (Fig. 1).

### Somatic Cell Count

Samples were decanted from BTM into 5 mL plastic tubes during morning milking daily for 1 month beginning on the first treatment day. Somatic cell counting was performed using a cell counting device (DeLaval Cell Counter®, DeLaval International, Sweden) (Dohoo, 2001; Harmon, 2001; Pyörala, 2003).

### Measurement of Milk Progesterone and Estradiol Levels

Samples were decanted from BTM into 5 mL glass tubes during morning milking on days 0, 4, 7, 10, 13, 16, 19, 22, 25, 28 and 31 beginning on the first treatment day. The samples were kept at -20°C until being analyzed. Estradiol and progesterone measurements were made using commercial ELISA kits (Bioassay Technology Laboratory USA) (Schams and Karg, 1986).

### Animals

A total of 100 cattle aged between 2 and 4 years were randomized into four equal groups of 25. Groups were matched for age and yield properties. All animals were in their 40–60<sup>th</sup> postpartum days at the beginning of treatment. Group 1 included untreated animals (control group). Animals in Group 2 were implanted with a PRID (Delta, Ceva). This device was kept in the vaginas for 7 days and those reaching estrus after its removal were bred. Animals in Group 3 were administered a double-dose of IM 25 mg dinoprost (Enzaprost®-T, Ceva) with an 11 day interval and those reaching estrus were bred. Animals in Group 4 underwent the Ovsynch protocol. For this purpose, cattle, which were in their postpartum 40–60<sup>th</sup> days, underwent administration of intramuscular 100 mcg gonadorelin diacetate tetrahydrate (Ovarelin, Ceva) on day 0, 25 mg dinoprost (Enzaprost®-T, Ceva) on the 7<sup>th</sup> day, and 100 mcg gonadorelin diacetate tetrahydrate (Ovarelin, Ceva) and fixed-time artificial insemination were carried out between 16–24<sup>h</sup>.

### Statistical Analysis

The Pearson's correlation coefficients between the milk SCC, progesterone and the estradiol values were calculated for each group using SPSS (version 22.0) (SPSS, 2015).

## Results

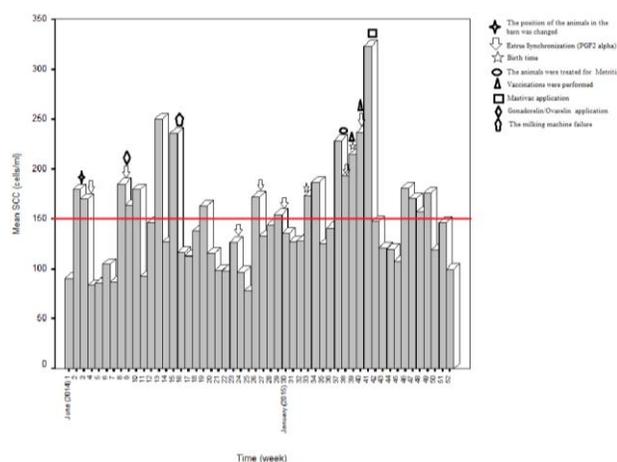
The milk progesterone, estradiol and SCC values and their correlations are shown in Table 1 and 2, respectively. The correlation coefficients between the BTMSCCs and progesterone levels in all groups were between 0.277 and 0.366, while the correlation coefficients between the BTMSCCs and estradiol levels were between -0.167 and 0.417. The correlation coefficients between BTMSCC and progesterone level in the control group (0.366) and between BTMSCC and estradiol levels in the Ovsynch group (0.417) were moderate but not statistically significant ( $P > 0.05$ ). The milk BTMSCCs values for the control and Ovsynch groups were relatively stable and lower than those of the PRID and PGF<sub>2</sub>-alpha groups (Fig. 2).

**Table 1:** The progesterone, estradiol and BTMSCC values of the milk versus control days obtained from the study and control groups

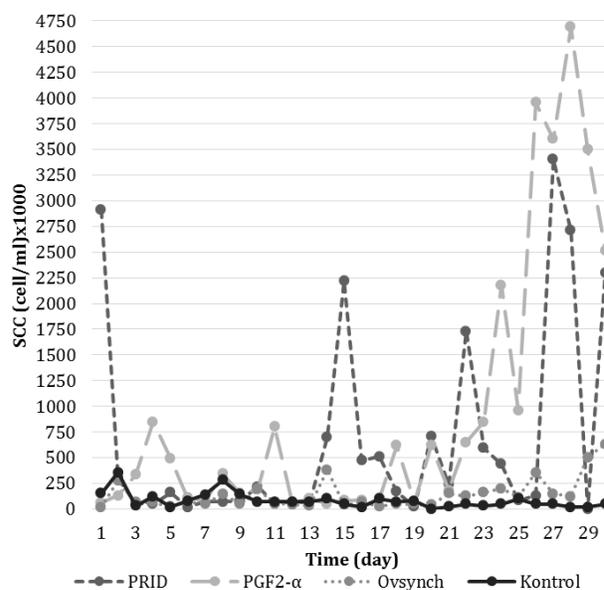
Groups	Days	Progesterone (ng/mL)	Estradiol (pg/mL)	SCC (cell/mL) x 1000
Control	0.	2.0	37.2	150
	4.	6.0	28.4	122
	7.	0.5	30.7	140
	10.	0.5	27.5	70
	13.	2.2	32.1	70
	16.	0.5	35.3	16
	19.	2.2	28.4	80
	22.	0.7	36.5	50
	25.	2.5	38.0	100
	28.	2.1	29.0	18
	31.	0.6	55.2	50
	0.	0.6	36.4	2,912
PRID	4.	2.1	31.7	49
	7.	4.7	59.4	70
	10.	0.5	58.9	219
	13.	0.5	31.1	60
	16.	4.1	59.3	473
	19.	2.6	36.5	21
	22.	1.8	49.6	1,732
	25.	2.2	29.0	85
	28.	4.1	29.5	2,712
	31.	2.5	44.2	2,296
	0.	6.0	50.1	60
	4.	2.6	53.3	847
PGF <sub>2</sub> -alpha	7.	5.8	36.0	77
	10.	2.0	27.7	190
	13.	1.2	49.8	102
	16.	0.8	51.3	86
	19.	4.5	36.4	61
	22.	0.5	35.3	649
	25.	4.9	31.2	962
	28.	2.6	50.0	4,690
	31.	2.5	27.0	2,515
	0.	0.5	45.4	20
	4.	2.1	48.3	65
	7.	5.0	43.1	50
Ovsynch	10.	0.7	33.6	197
	13.	4.1	39.3	29
	16.	2.6	37.3	67
	19.	4.0	40.2	50
	22.	1.5	50.4	127
	25.	2.4	50.3	114
	28.	0.6	44.8	123
	31.	4.8	52.7	632

**Table 2:** The correlation coefficients (r) and significances (p) between the progesterone, estradiol and BTMSCC values of the milk obtained from the study groups formed by different synchronization methods

BTMSCC	Progesterone ng/mL (n=11)	Estradiol pg/ml (n=11)
control (n=11)	r 0.366	-0.146
	p 0.269	0.669
PRID (n=11)	r -0.020	-0.167
	p 0.953	0.624
PGF <sub>2</sub> -alfa (n=11)	r -0.131	0.041
	p 0.702	0.904
Ovsynch (n=11)	r 0.277	0.417
	p 0.410	0.202



**Fig. 1:** Distribution of BTMSCC according to weeks. \* cells/mLx1000 (The data were obtained from the pre-published study named “Microbiological quality within the bulk milk tank and management practices in a dairy farm with low somatic cell count in Turkey”)



**Fig. 2:** The BTMSCC values of the milk obtained from the control and the study groups according to the sampling

### Discussion

Estrus synchronization methods have been frequently used in large dairy cattle farms to simplify care and feeding. However, enhancing breeding profit is the primary objective of such approaches and udder health and milk yield are generally neglected. Here we aimed to investigate the effects of various estrus synchronization methods on BTMSCC and to identify the method having the least impact on BTMSCC.

Significant information on udder health, milk quality of a herd and the herd itself can be gained by monitoring BTMSCC. By considering BTMSCC data over a 3 month period, it is possible to obtain data on milk capacity loss, the prevalence of subclinical mastitis, the duration and severity of the infection and whether the causative microorganism is environmental or contagious, as well as the management of mammary health or health of the herd prior to and after birth. However, these data should be interpreted cautiously, as multiple factors can affect SCC and BTMSCC.

The estrus periods of the animals can affect BTMSCC. The increase in these periods should be benefited from, and should be counteracted in the shortest possible time. Thus, it is important to choose the method to minimally affect the BTMSCC in estrus synchronizations. Currently, the number of studies evaluating the effects of estrus synchronization protocols in practice on the BTMSCCs in the dairy farms is limited. However, the breeding capacity parameters are commonly considered when applying these estrus protocols rather than the mammary health (Bastan, 2013).

It has been suggested that mastitis reduces fertility parameters in cattle and, therefore, that fertility parameters may be recovered by reducing the incidence of mastitis, thereby increasing reproduction rates. Elevated SCC is considered a useful indicator of subclinical mastitis (Hudson et al., 2012). Lavon et al. (2011) have reported that high SCC levels during artificial insemination may be related to reduce pregnancy rates and that pregnancy rates increase with decreased levels of SCC, an indicator of subclinical mastitis. McDougall and Voermans (2002) attributed the decrease in milk yield during estrus to an increased SCC level in goats when using two different estrus synchronization methods. Lavon et al. (2016) reported pregnancy rates in cattle treated with the Ovsynch protocol that were similar to cattle without mastitis. Based on this finding, the Ovsynch protocol was proposed as a means of increasing fertility in cattle with subclinical mastitis. In line with this, here we found that, among the synchronization methods tested, the Ovsynch protocol least increased BTMSCC, which was not significantly different from the untreated group.

Based on our results, we propose that farms with large numbers of cattle either avoid synchronization of sexual cycles or using the Ovsynch method. Such approaches would be expected to benefit udder health.

## Acknowledgements

This study was supported by the TUBITAK - 215O578.

## References

- Aslan, S. and A. Gumen, 2012. Fertility control programs. In: *Obstetric and Gynecology in Farm Animals*, pp: 469–517. Semacan, A., M. Kaymaz, M. Findik, A. Risvanli, A. Koker (eds.). Medipres, Malatya, Turkey
- Barkema, H.W., S. De Vliegher, S. Piepers and R.N. Zadoksc, 2013. Herd level approach to high bulk milk somatic cell count problems in dairy cattle. *Vet. Quart.*, 33: 82–93
- Bastan, A., 2013. *Udder Health and Problems in Cattle*, pp: 173–183. Kardelen Ofset, Ankara, Turkey
- Bastan, A., M. Kaymaz, M. Findik and N. Erunal, 1997. The use of electrical conductivity, somatic cell count and california mastitis test in diagnosis of subclinical mastitis in dairy cows. *Ankara Univ. Vet. Fak.*, 44: 1–5
- Busato, A., P. Trachsel, M. Schallibaum and J.W. Blum, 2000. Udder health and risk factors for subclinical mastitis in organic dairy farms in Switzerland. *Prev. Vet. Med.*, 28: 205–220
- Deveci, H., A.M. Apaydin, C. Kalkan and H. Ocal, 1994. *Udder Diseases in Domestic Animals*, pp: 123–129. Univ Firat Press, Elazığ, Turkey
- Dohoo, I.R., 2001. Setting SCC cutpoints for cow and herd interpretation. *Proceedings of the National Mastitis Council 40<sup>th</sup> Annual Meeting*, pp 10–18. USA
- Furumura, K., M. Imanishi, F. Kashiwamura, Y. Shinde, S. Kawabata and M. Hayashi, 1995. On-line image processing prototype for the detection of mastitis in cows. *J. Anim. Sci. Technol.*, 66: 882–888
- Gillespie, B.E., M.J. Lewis, S. Boonyayatra, M.L. Maxwell, A. Saxton, S.P. Oliver and R.A. Almeida, 2012. Evaluation of bulk tank milk microbiological quality of nine dairy farms in Tennessee. *J. Dairy Sci.*, 95: 4275–4279
- Harmon, R.J., 1994. Physiology of mastitis and factors affecting somatic cell counts. *J. Dairy Sci.*, 77: 2103–2112
- Harmon, R.J., 2001. Somatic cell counts. *Proc. National Mastitis Council Annual Meeting Proceeding*, pp: 3–9. Lexington, Kentucky USA
- Hudson, C.D., A.J. Bradley, J.E. Breen and M.J. Green, 2012. Associations between udder health and reproductive performance in United Kingdom dairy cows. *J. Dairy Sci.*, 95: 3683–3697
- Lavon, Y., E. Ezra, G. Leitner and D. Wolfenson, 2011. Association of conception rate with pattern and level of somatic cell count elevation relative to time of insemination in dairy cows. *J. Dairy Sci.*, 94: 4538–4545
- Lavon, Y., M. Kaim, G. Leitner, D. Biran, E. Ezra and D. Wolfenson, 2016. Two approaches to improve fertility of subclinical mastitic dairy cows. *J. Dairy Sci.*, 99: 2268–2275
- McDougall, S. and M. Voermans, 2002. Influence of estrus on somatic cell count in dairy goats. *J. Dairy Sci.*, 85: 378–383
- Macmillan, K.L., 2010. Recent advances in the synchronization of estrus and ovulation in dairy cows. *J. Reprod. Dev.*, 56: 42–47
- Pyörälä, S., 2003. Indicators of inflammation in the diagnosis of mastitis. *Vet. Res.*, 34: 565–578
- Schams, D. and H. Karg, 1986. Hormones in milk. *Ann. NY Acad. Sci.*, 464: 75–86
- Semacan, A. and S.M. Pancarci, 2012. Management of reproduction. In: *Obstetric and Gynecology in Farm Animals*, pp: 99–124. Semacan, A., M. Kaymaz, M. Findik, A. Risvanli and A. Koker (eds.). Medipres, Malatya, Turkey
- Smith, K.L., 1996. Standards for somatic cells in milk: Physiological and regulatory. *Mastitis Newsletter, Newsletters IDF*, 144: 7–9
- Stevenson, J.S., 2016. Synchronization and artificial insemination strategies in dairy herds. *Vet. Clin. North Am. Food. Anim. Pract.*, 32: 349–364
- SPSS, 2015. *SPSS 22.0 Version, Statistical Package in Social Sciences for Windows*. Chicago, USA

(Received 01 February 2017; Accepted 19 April 2017)