

Effects of the post-partum period on *in vivo* embryo production in Simmental cows

Elif Merve Çınar¹, Mehmet Ferit Özmen¹, İbrahim Küçükcaslan², Mehmet Köse², Erkan Say^{3*}, Ümüt Cirit³

¹ Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Dicle University, Diyarbakir, Türkiye; ² Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Dicle University, Diyarbakir, Türkiye; ³ Department of Reproduction and Artificial Insemination, Faculty of Ceyhan Veterinary Medicine, Çukurova University, Adana, Türkiye.

Article Info	Abstract
Article history: Received: 02 February 2025 Accepted: 20 May 2025 Available online: 15 February 2026	The aim of this study was to investigate the effects of post-partum (PP) periods of different lengths on super-stimulatory and super-ovulatory responses, as well as the number and quality of embryos in Simmental cows. Fifty cows were divided into five groups based on their PP days, including 50 - 60 days (n = 5), 61 - 90 days (n = 17), 91 - 120 days (n = 9), 121 - 150 days (n = 9), and 151 - 420 days (n = 10). On a random day of the estrous cycle, all follicles larger than 5.00 mm on the ovaries were aspirated and a progesterone-releasing device was inserted into the vagina of all donors. Approximately 36 hr after follicle aspiration, all cows were administered 500 µg of follicle-stimulating hormone in decreasing doses, twice daily for 6 days. Ovaries were evaluated using trans-rectal ultrasonography during human chorionic gonadotropin treatment and after embryo collection to assess follicles and corpora lutea, respectively. Super-stimulatory and super-ovulatory responses, as well as embryo yield and quality were found to be similar among cows in the PP days groups of 61 - 90, 91 - 120, 121 - 150, and 151 - 420. However, the embryo recovery rate and mean number of transferable and freezable embryos were adversely affected in cows at 50 - 60 days PP. The findings of our study suggested that day 60 PP served as a threshold for <i>in vivo</i> embryo production in Simmental cows.
Keywords: Cow Embryo <i>In vivo</i> Simmental Super-ovulation	

© 2026 Urmia University. All rights reserved.

Introduction

In developing countries, like Türkiye, where a significant portion of the population relies on agriculture both directly and indirectly, it is of great importance to promote the use of reproductive biotechnology techniques, such as embryo transfer, in the field to enhance livestock productivity.^{1,2} Although Türkiye was the second country in the world to introduce artificial insemination, unfortunately, embryo transfer is not widely used in the field.² Despite highly successful scientific studies being conducted,³⁻⁷ the number of embryo transfers is still not at a level that can be compared to European countries or other countries with advanced livestock industries. It is encouraging that in recent years, the establishment of high-capacity professional dairy farms has been steadily increasing interest in embryo transfer.²

Embryo transfer is a crucial biotechnological method, enabling rapid genetic improvement within a herd.¹ However, there are numerous factors that affect the

success of *in vivo* embryo production.⁸ One of the key questions is how donors will individually respond to super-stimulator treatment.¹ Another important question is when the optimal period for *in vivo* embryo production occurs. Because delaying the super-stimulatory treatment prolongs the calving interval of the donor. Similarly, starting *in vivo* embryo production too early after calving may negatively affect oocyte and embryo qualities and the total number of embryos produced for various reasons.⁹ Donor animals are usually placed on a super-ovulatory schedule at least 45 days after calving.¹⁰ During the post-partum (PP) period in cows, several processes take place, including uterine involution, myometrial contractions, expulsion of lochia (fetal membranes), endometrial regeneration, resumption of cyclic ovarian activity, and elimination of bacteria from the uterine lumen. By 46 days PP, cytological examinations indicated that nearly 40.00% of cows still had unresolved uterine inflammation which negatively impacted their pregnancy rates and caused more pregnancy losses.¹¹ Sub-clinical endometritis, being identified based on the percentage of polymorphonuclear

*Correspondence:

Erkan Say. PhD

Department of Reproduction and Artificial Insemination, Faculty of Ceyhan Veterinary Medicine, Çukurova University, Adana, Türkiye

E-mail: esay@cu.edu.tr



This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International (CC BY-NC-SA 4.0) which allows users to read, copy, distribute and make derivative works for non-commercial purposes from the material, as long as the author of the original work is cited properly.

cells in endometrial smears at the onset of super-stimulation, was reported to reduce the fertilization rate of oocytes.¹² It was also shown that experimentally induced endometritis reduced the capacity of oocytes to develop to morulae following *in vitro* fertilization.¹³

In lactating cows, PP negative energy balance (NEB) stands out as the most critical nutritional factor influencing fertility, and consequently, embryo production.⁹ The increased nutritional demand, coupled with suppressed appetite, typically leads dairy cows into a state of NEB during the last weeks of pregnancy and the first two months after calving.¹⁴ The follicular fluid composition during the NEB period mirrors systemic alterations in various metabolites and metabolic hormones.⁹ Cows experiencing NEB have increased blood concentrations of non-esterified fatty acids, urea, and β -hydroxybutyrate along with reduced circulating levels of glucose and insulin-like growth factor-I.^{9,15,16} Consequently, these adverse conditions are harmful to the oocyte, reducing its developmental competence.⁹

The effects of the PP period on embryo quality and *in vivo* embryo production in cattle have mostly been studied in dairy cows, and no consensus has been reached on this matter. Studies have mostly reported that the PP period has no effect on *in vivo* embryo production in dairy cows,¹⁷⁻²² while some researchers have suggested a significant effect.²³⁻²⁵ Moreover, most studies on this topic have focused on standard characteristics, such as the number and quality of embryos. Super-ovulation treatments in dairy cows are generally initiated after the 60th day PP.⁹ However, as far as we know, there are no available data on the optimal time for initiating super-stimulation in Simmental cows after calving.

For these reasons, this study aimed to investigate the effect of PP periods of different lengths on super-stimulatory and super-ovulation responses as determined by the number of follicles and corpora lutea, respectively, as well as the number and quality of embryos in Simmental cows.

Materials and Methods

All procedures performed in the study were approved by the Dicle University Local Ethics Committee for Animal Experiments, Diyarbakir, Türkiye (Approval No. 92406). The study was carried out at the TIGEM Ceylanpınar Agricultural Enterprise Directorate, Şanlıurfa, Türkiye, between March and June 2018, in four replicates. Cows with a body weight of 500 to 650 kg, a body condition score between 3.00 and 4.00 (on a 1.00 - 5.00 scale), and a PP period of ≥ 50 days were utilized. The cows were kept under the same environmental, management, and feeding conditions. Cows with a history of abortion and those diagnosed with endometritis within the last month were not included in the study. The gynecological condition of

all cows was assessed prior to the commencement of the study and cows with asymmetry and abnormal firmness in the uterine horns or abnormal vaginal discharge were not included in the study. A total number of 50 cows were categorized into five groups according to the days PP, including 50 - 60 days (n = 5), 61 - 90 days (n = 17), 91 - 120 days (n = 9), 121 - 150 days (n = 9), and 151 - 420 days (n = 10).

Follicular aspiration (FA) and super-stimulation.

On a random day of the estrous cycle, the ovaries of each cow were examined trans-rectally using ultrasound (Esaote Pie Medical Aquila, Istanbul, Türkiye). All follicles larger than 5.00 mm on the ovaries were aspirated trans-vaginally using an 18-gauge disposable needle and a 7.50 MHz convex probe-equipped ultrasound (Nutricell/Esaote-Pie Medical, Campinas, Brazil). Before FA, an epidural anesthesia using 5.00 - 7.00 mL of 2.00% lidocaine (Vilsan, Ankara, Türkiye) was administered to each cow to reduce peristalsis and discomfort. Following FA, a progesterone-releasing device (1.38 g progesterone; Zoetis Animal Health, Istanbul, Türkiye) was inserted into the vagina. Approximately 36 hr after FA, all cows were administered a 500 μ g of follicle-stimulating hormone (FSH; Reprobiol SPRL, Ouffet, Belgium) in decreasing doses (75.00, 65.00, 50.00, 50.00, 40.00, 40.00, 35.00, 35.00, 30.00, 30.00, 25.00, and 25.00 μ g) twice daily over 6 days for the purpose of super-stimulation.⁴ The cows received a single intramuscular dose of 400 IU equine chorionic gonadotropin (MSD Animal Health, Istanbul, Türkiye) 24 hr prior to the initiation of FSH treatments.⁴ All donors were treated with 25.00 mg prostaglandin F2 α (Zoetis Animal Health) intramuscularly alongside the 9th and 10th FSH administrations. Intra-vaginal devices were withdrawn at the time of the final FSH administration. Twenty-four hr following progesterone-releasing device removal, ovulations were triggered by intramuscular administration of 1,500 IU human chorionic gonadotropin (MSD Animal Health). Artificial inseminations were carried out three times at 12, 24, and 36 hr following human chorionic gonadotropin administration, utilizing pre-evaluated frozen semen from two separate bulls. All cows received prostaglandin F2 α at the same time as the first artificial inseminations. Each cow ovary was evaluated using trans-rectal ultrasonography during human chorionic gonadotropin treatment to assess the number and size of follicles. A positive response to the super-stimulatory treatment was defined as the presence of ≥ 3 ovulatory follicles measuring 9.00 mm or larger.

Ova/embryo collection. Each donor was epidurally anaesthetized (5.00 to 7.00 mL 2.00% lidocaine) before uterus flushing. Ova/embryo collection was performed non-surgically between 6.50 and 7 days following the first artificial inseminations, utilizing lactated Ringer's solution

supplemented with 1.00% calf serum (Sigma-Aldrich, St. Louis, USA) and 125 mg L⁻¹ kanamycin (Vetaş, İstanbul, Türkiye)³ which 800 - 1,000 mL of the solution was used to flush each uterine horn. Flushing was performed using a two-way Foley catheter in combination with the interrupted-syringe technique. The aspirates were transferred into embryo collection filters (Şark Kemikal, İstanbul, Türkiye). The collected oocytes and/or embryos were assessed for developmental stage and quality at 50.00 × magnification based on the criteria established by the International Society for Embryo Technology.²⁶ Embryos were categorized as transferable (grades 1, 2, and 3) and freezable (grades 1 and 2).²⁷ The ovaries were assessed using trans-rectal ultrasonography to evaluate the presence and quantity of corpus luteum (CL) following embryo collection. In donors with three or more CLs detected, the super-ovulatory response was considered positive.²⁸

Statistical analysis. Statistical analyses were conducted using SPSS Software (version 10.0; SPSS Inc., Chicago, USA). It was verified using the Shapiro-Wilks test whether the data were normally distributed. Since the data distribution was not normal, the numbers of CL, follicles (small and large), ova (fertilized and unfertilized), embryos (total, degenerate, transferable, and freezable), and proportional data were compared using the Mann-Whitney U test. The results were presented as the mean ± standard error of the mean and deemed statistically significant when $p < 0.05$.

Table 1. Comparison of small (< 9.00 mm) and ovulatory (≥ 9.00 mm) follicle counts, as well as the super-stimulatory response among the study groups.

Post-partum interval (days) groups	50 - 60	61 - 90	91 - 120	121 - 150	151 - 420
Number of cows	5	17	9	9	10
Number of follicles ≥ 9.00 mm	15.60 ± 4.38	15.47 ± 1.86	11.67 ± 4.59	13.44 ± 1.17	21.80 ± 3.88
Number of follicles < 9.00 mm	3.60 ± 0.93	3.06 ± 0.58	2.89 ± 0.72	2.11 ± 0.51	3.10 ± 0.80
Total number of follicles	19.20 ± 5.06	18.53 ± 2.00	14.56 ± 2.82	15.56 ± 1.31	24.90 ± 3.81
Super-stimulatory response (%)	100	100	100	100	100

^{ab} Within rows, means with no common letters are statistically different ($p < 0.05$).

Table 2. Comparison of embryo yield and super-stimulatory response among the study groups.

Post-partum interval (days) groups	50 - 60	61 - 90	91 - 120	121 - 150	151 - 420
Number of cows	5	17	9	9	10
Number of CL on the day of embryo collection	21.20 ± 2.92	19.91 ± 2.46	14.73 ± 2.29	19.70 ± 2.40	23.69 ± 3.71
Super-ovulatory response (%)	100	100	100	100	100
Anovulatory follicles	2.40 ± 0.24	1.80 ± 0.34	1.29 ± 0.57	2.00 ± 0.93	1.80 ± 0.59
Recovery rate (%) [*]	20.20 ^a	56.4 ^b	59.10 ^b	54.60 ^b	54.20 ^b
Total ova/embryos recovered	4.21 ± 2.13	12.00 ± 2.07	8.14 ± 2.11	9.63 ± 1.65	13.00 ± 3.40
Unfertilized ova	0.00 ± 0.00	0.80 ± 0.39	1.86 ± 1.32	0.13 ± 0.13	2.10 ± 1.49
Fertilized ova	4.20 ± 2.13	11.20 ± 1.88	6.29 ± 1.78	9.50 ± 1.57	10.90 ± 2.18
Fertilized ova (%)	100	94.41	78.15	99.27	92.40
Degenerate embryos	1.60 ± 0.68	2.53 ± 0.76	0.71 ± 0.57	1.38 ± 0.32	4.10 ± 1.64
Transferable embryos (grades 1 - 3)	2.60 ± 1.47 ^a	8.53 ± 1.49 ^b	5.57 ± 1.80 ^{ab}	8.00 ± 1.76 ^{ab}	6.70 ± 1.37 ^{ab}
Transferable embryos (%)	55.33	75.41	71.93	77.42	59.39
Freezable embryos (grade 1)	0.60 ± 0.40 ^a	7.40 ± 1.40 ^b	5.00 ± 1.69 ^{ab}	7.13 ± 1.74 ^b	5.80 ± 1.33 ^b
Freezable embryos (%)	22.33 ^a	66.44 ^b	64.78 ^b	67.26 ^b	49.74 ^{ab}

^{*} Proportion of ova/embryos recovered over the number of corpora lutea (CL) counted.

^{ab} Within rows, means with no common letters are statistically different ($p < 0.05$).

Results

The numbers of small, large, and total follicles, as well as the super-stimulator response were found to be similar among the groups ($p > 0.05$; Table 1). All animals included in the study responded to the super-ovulation treatment (Table 2). The numbers of CL, anovulatory follicles, total ova/embryos recovered, fertilized and unfertilized ova, and degenerated embryos, as well as the percentages of super-ovulatory response and fertilized ova were similar among the groups ($p > 0.05$). The recovery rate of cows in the PP 50 - 60 days group was found to be significantly lower than that of cows in all other groups ($p < 0.05$). The cows in the PP 50 - 60 days group had the lowest number of transferable embryos and the difference between this group and the PP 61 - 90 days group was found to be significant ($p < 0.05$). Similarly, the number of freezable embryos in the cows of the PP 50 - 60 days group was found to be significantly lower than that of cows in all other groups except the PP 91 - 120 days group ($p < 0.05$). The cows in the PP 61 to 420 days range were found to have similar values for all parameters examined in the study.

Discussion

The relationship between the PP period and embryo quality and production in cattle can be assessed through correlation analysis, reflecting general trends, or through more specific evaluations where donors are grouped

according to their PP periods. Although correlation analyses provide general trends, they do not offer specific information about the most suitable or unsuitable periods for embryo production in cows. The general trend in studies suggests that the PP period does not have an effect on *in vivo* embryo production in dairy cows.¹⁷⁻²² However, some studies have reported that the PP period has a significant effect on *in vivo* embryo production.²³⁻²⁵ One reason for this difference in opinion could be the use of different evaluation methods. Another reason might be that, particularly in studies where cows are grouped according to the specific PP periods, the classification of PP periods may differ between studies. Another notable aspect is that the majority of studies in this area have been conducted on dairy breeds (particularly Holstein) and there is a lack of data regarding combined or beef breeds. There is only one study suggesting a positive relationship between PP period and the total number of transferable embryos in Simmental cows, a dual purpose breed.⁵ However, in this study, apart from the information that the average PP period was 126 days, there was no information available about the distribution of PP periods in cows (*e.g.*, earliest and latest).⁵ To the best of our knowledge, the present study was the first detailed experiment in which Simmental cows were grouped according to their PP periods to evaluate *in vivo* embryo production. In this study, the super-stimulator (small and large follicle counts) and super-ovulator (CL counts) responses were found to be similar among all five PP period groups. However, the recovery rate of cows in the PP 50 - 60 days group was found to be significantly lower than that of all other groups. Although the numbers of large follicles (≥ 9.00 mm) were developed after super-stimulation and the numbers of CLs after super-ovulation were found to be similarly high in the PP 50 - 60 days group compared to all other groups, the lower recovery rate and numerically lower total ova/embryos recovered suggested that the uterine environment of Simmental cows at 50 to 60 days PP might not be very suitable for flushing procedures. These data suggested that in some cows at 50 - 60 days PP, uterine involution might not have been fully completed, which could have negatively affected the number of embryos recovered. Overall, the lowest recovery rate, number of transferable and freezable embryos, and percentage of freezable embryos were obtained from cows at PP days 50 - 60. Walters *et al.*²⁹ reported that dairy cows produced a higher percentage of good-quality oocytes on day 119 PP compared to cows on day 32 PP. Similarly, Kendrick *et al.*³⁰ found that the numbers of oocytes were increased linearly from PP day 30 to day 100.

Heightened nutrient requirements linked to decreased appetite typically lead dairy cows into a state of NEB, being commonly seen during the final week of pregnancy and the first two months after calving.¹⁴ During the NEB phase, alterations in various metabolites and metabolic

hormones at the systemic level are mirrored in the follicular fluid. Characteristic changes, including increased levels of non-esterified fatty acids or β -hydroxybutyrate, along with reduced glucose availability during *in vitro* oocyte maturation, negatively affect the oocyte, compromising its developmental potential and early embryo formation.⁹ It was reported that the metabolic changes in Simmental cows during the peri-parturient period were less pronounced compared to those in Holstein-Friesian cows.³¹ However, the lower embryo yield in cows at PP days 50 - 60 suggests that NEB may have a negative effect in Simmental cows as well. Similarly, Erdem *et al.*⁵ suggested that the positive correlation they found between the PP period and counts of total CL, total oocyte/embryo, transferable embryo, and code I embryo might have been due to the improvements in NEB, ovarian activity, and uterine health as days in milk progressed. However, there is a lack of information regarding the relationship between NEB and *in vivo* embryo production in Simmental cows, and further studies are needed.

Numerically, the highest fertilized ova rates as well as transferable and freezable embryo numbers were obtained from the PP 61 - 90 days group. Moreover, the transferable and freezable embryo numbers in this group were significantly higher than those in the PP 50 - 60 days group. Sahara *et al.*²³ reported that the mean numbers of transferable embryos were 3.00, 4.00, 12.00, 3.50, and 3.00 for PP days groups of 42 - 49, 63 - 70, 84 - 91, 105 - 112, and 126 - 133, respectively, in Japanese Black cows. Similar to the findings of the present study, the highest numbers of recovered and transferable embryos were observed on PP days 84 - 91.²³

According to Isogai *et al.* the numbers of normal embryos were decreased linearly from 131 - 190 days to ≥ 461 days PP, with more normal embryos found in Holstein cows at 70 - 130, 131 - 190, and 191 - 250 days compared to the cows at ≥ 461 days.²⁴ In this study, all parameters examined (*e.g.*, super-stimulatory and super-ovulatory responses and embryo yield and quality) were found to be similar among cows in the PP days groups of 61 - 90, 91 - 120, 121 - 150, and 151 - 420. Similarly, Lim¹⁹ found that the numbers of transferable embryos in Holstein cows at PP days of 61 - 90, 91 - 120, 121 - 150, and ≥ 151 were similar (10.70, 6.80, 9.80, and 5.70, respectively).

It was concluded from the study that the embryo recovery rate and mean numbers of transferable and freezable embryos in cows at 50 - 60 days PP were adversely affected; therefore, waiting until day 60 PP is recommended for *in vivo* embryo production in Simmental cows.

Acknowledgments

This study was carried out within the scope of embryo transfer R & D study protocol for combined breed cattle

production signed between TIGEM and Dicle University, Diyarbakir, Türkiye. The authors would like to thank Prof. Dr. Ömür Koçak for statistical analysis.

Conflict of interest

The authors declare no competing interest.

References

- Mahmood K, Tahir MZ, Butt MA, et al. GnRH or estradiol benzoate combination with CIDR improves *in-vivo* embryo production in bovines (*Bos indicus* and *Bos taurus*) under subtropics. PeerJ 2021; 9: e12077. doi: 10.7717/peerj.12077.
- Sağirkaya H. Embryo transfer practice in cattle and its importance for Türkiye [Turkish]. Uludag Univ J Fac Vet Med 2009; 28(2): 11-19.
- Karaşahin T, Akyol N, Satılmış M, et al. Investigation of conception rates achieved with the transfer of sexed and unsexed bovine embryos. Turk J Vet Anim Sci 2014; 38(3): 253-256.
- Cirit Ü, Özmen MF, Köse M, et al. Effects of presence or absence of a dominant follicle estimated by a single ultrasound examination at the time of follicular aspiration on superovulatory responses and embryo production in lactating Simmental cows. Kafkas Univ Vet Fak Derg 2019; 25(5): 627-632.
- Erdem H, Alkan H, Karaşahin T, et al. Retrospective evaluation of factors affecting superovulatory response in embryo production in Simmental cattle. Turk J Vet Anim Sci 2020; 44(6): 1250-1259.
- Kara U, Bekyürek T. The effects of pre-superovulation GnRH and short-term progesterone administrations on the quantity and quality of bovine embryos. Erciyes Üniv Vet Fak Derg 2021; 18(1): 41-47.
- Koca D, Aktar A, Turgut AO, et al. The effect of conventional semen, sexed-semen, and embryo transfer on pregnancy rate in Holstein dairy cows. J Res Vet Med 2023; 42(2): 99-103.
- Jahnke MM, Youngs CR. Superovulation in cattle. In: Hopper RM (Ed). Bovine Reproduction 2nd ed. Auburn, AL, USA: John Wiley & Sons, Inc., 2021; 1032-1040.
- Mikkola M, Hasler JF, Taponen, J. Factors affecting embryo production in superovulated *Bos taurus* cattle. Reprod Fertil Dev 2020; 32(2): 104-124.
- Phillips PE, Jahnke MM. Embryo transfer (techniques, donors, and recipients). Vet Clin North Am Food Anim Pract 2016; 32(2): 365-385.
- Lima FS, Bisinotto RS, Ribeiro ES, et al. Effects of 1 or 2 treatments with prostaglandin F₂α on subclinical endometritis and fertility in lactating dairy cows inseminated by timed artificial insemination. J Dairy Sci 2013; 96(10): 6480-6488.
- Carvalho PD, Souza AH, Sartori R, et al. Effects of deep-horn AI on fertilization and embryo production in superovulated cows and heifers. Theriogenology 2013; 80(9): 1074-1081.
- Dickson MJ, Piersanti RL, Ramirez-Hernandez R, et al. Experimentally induced endometritis impairs the developmental capacity of bovine oocytes. Biol Reprod 2020; 103(3): 508-520.
- Bisinotto RS, Greco LF, Ribeiro ES, et al. Influences of nutrition and metabolism on fertility of dairy cows. Anim Reprod 2012; 9(3): 260-272.
- Leroy JL, Vanholder T, Mateusen B, et al. Non-esterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes *in vitro*. Reproduction 2005; 130(4): 485-495.
- Leroy JL, Vanholder T, Opsomer G, et al. The *in vitro* development of bovine oocytes after maturation in glucose and beta-hydroxybutyrate concentrations associated with negative energy balance in dairy cows. Reprod Domest Anim 2006; 41(2): 119-123.
- Darrow MD, Lindner GM, Goemann GG. Superovulation and fertility in lactating and dry dairy cows. In Proceedings: Eighth Annual Conference of the International Embryo Transfer Society. Denver, Colorado, USA; 84.
- Hasler JF, McCauley AD, Schermerhorn EC, et al. Superovulatory responses of Holstein cows. Theriogenology 1983; 19(1): 83-99.
- Lim KT. Effects of milk production, postpartum days or seasons on *in vivo* embryo production by superovulation in holstein cows. K J Emb Trans 2009; 24(1): 33-37.
- Lee W, Song K, Lim K, et al. Influence of factors during superovulation on embryo production in Korean Holstein cattle. J Vet Med Sci 2012; 74(2): 167-174.
- Hussein MM, Abdel Aziz RL, Abdel-Wahab A, et al. Preliminary study of factors affecting the superovulatory response of high producing dairy cows superstimulated regardless of the stage of estrous cycle in Egypt. Beni-Suef Univ J Basic Appl Sci 2014; 3(4): 286-292.
- Ferraz PA, Burnley C, Karanja J, et al. Factors affecting the success of a large embryo transfer program in Holstein cattle in a commercial herd in the southeast region of the United States. Theriogenology 2016; 86(7): 1834-1841.
- Sahara H, Shimura O, Kawato Y, et al. Timing of superovulation treatments in postpartum Japanese Black cows. Jpn J Anim Reprod 1991; 37(1): 33-36.
- Isogai T, Shimohira I, Kimura K. Factors affecting embryo production following repeated superovulation treatment in Holstein donors. J Reprod Dev 1993; 39(1): 79-84.
- Stádnik L, Bezdiček J, Makarevich A, et al. Ovarian activity and embryo yield in relation to the postpartum period in superovulated dairy cows. Acta Vet Brno

- 2017; 86(1): 51-57.
26. IETS. Manual of the International Embryo Transfer Society. 4th ed. Champaign Illinois: 2010.
 27. García Guerra A, Tribulo A, Yapura J, et al. Lengthening the superstimulatory treatment protocol increases ovarian response and number of transferable embryos in beef cows. *Theriogenology* 2012; 78(2): 353-360.
 28. Souza AH, Carvalho PD, Rozner AE, et al. Relationship between circulating anti-Müllerian hormone (AMH) and superovulatory response of high-producing dairy cows. *J Dairy Sci* 2015; 98(1): 169-178.
 29. Walters AH, Bailey TL, Pearson RE, et al. Parity-related changes in bovine follicle and oocyte populations, oocyte quality, and hormones to 90 days postpartum. *J Dairy Sci* 2002; 85(4): 824-832.
 30. Kendrick KW, Bailey TL, Garst AS, et al. Effects of energy balance on hormones, ovarian activity, and recovered oocytes in lactating Holstein cows using transvaginal follicular aspiration. *J Dairy Sci* 1999; 82(8): 1731-1741.
 31. Adamski M, Kupczyński R, Chladek G, et al. Influence of propylene glycol and glycerin in Simmental cows in periparturient period on milk yield and metabolic changes. *Arch Tierz* 2011; 54(3): 238-248.