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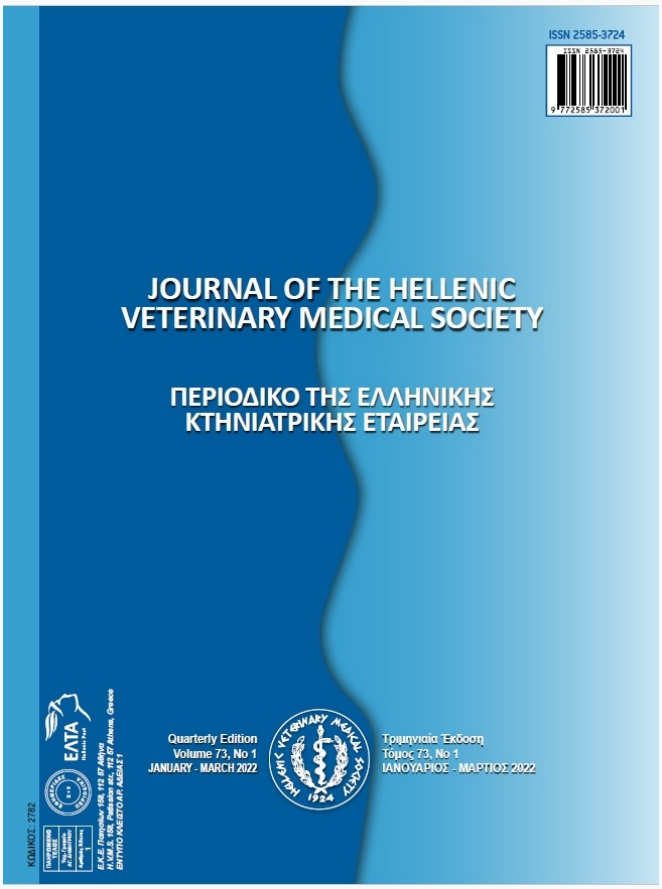

Effect of the locational relationship between the 1st wave dominant follicle and the corpus luteum on conception rate after embryo transfer: data analysis of 297 embryo transfers from a commercial embryo production program

Akira Goto, Koh Hayama, Manami Urakawa, Yoshio Oono, Ken Hazano, Mitsunori kayano, Shingo Haneda, Ken Nakada, Motozumi Matsui

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Research article
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Effect of the locational relationship between the 1st wave dominant follicle and the corpus luteum on conception rate after embryo transfer: data analysis of 297 embryo transfers from a commercial embryo production program

A. Goto[1], K. Hayama[2], M. Urakawa[2], Y. Oono[2], K. Hazano[1], M. Kayano[3], S. Haneda[1], K. Nakada[4], M. Matsui[1]**

[1]Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan

[2]Zen-noh Embryo Transfer Center, Kamishihoro, Hokkaido, Japan

[3]Research Center for Global Agromedicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan

[4]Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

ABSTRACT: The ovarian structures identified at the time of ET are important for the selection of recipients. In previous report, the first-wave dominant follicle, located ipsilateral to the corpus luteum on the ovary, was associated with reduced conception rate after artificial insemination. Thus, a similar locational relationship may affect conception rate during embryo transfer. Data from 297 transfers of fresh embryos to virgin heifers were analyzed aiming to check the effect of first-wave dominant follicle location in relation to the corpus luteum on conception rate using a multivariable logistic regression model with six confounders.The location of the first-wave dominant follicle in relation to the corpus luteum location had no significant effect on conception rate, suggesting that it is not necessary to consider the first-wave dominant follicle size and location in the ovaries for recipient selection.

***Keywords*:** corpus luteum; embryo transfer; first-wave dominant follicle; ipsilateral; recipient

Corresponding Author:
Motozumi Matsui, Department of Veterinary Medicine, Obihiro University of Agriculture and VeterinaryMedicine, Nishi-2-11, Inada-cho, Obihiro, Hokkaido, 080-8555, Japan
E-mail address: mmatsui@obihiro.ac.jp

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INTRODUCTION

Since the 1990s in Japan, the production of Japanese Black (JB; otherwise known as Wagyu) via embryo transfer (ET) in dairy cattle has rapidly increased. Since the demand for meat fromJB is increasing annually in Japan and abroad, JB beef has recently positioned as an important export resource for Japan. Currently, 100, 000 JB embryos are annually being transferred to recipients in Japan (MAFF, 2016). As a result, about 8% of JB calves are produced annually through ET, which also supports the demand for JB calves (Oro, 2019). However, during the past 20 years, the conception rate (CR) of ET in Japan has remained stagnant at 51% for fresh embryos and 45% in frozen embryos (MAFF, 2016). Further improvement of CR associated with ET is important to allow a more efficient production management of JB cattle.

Recipient selection is an important factor affecting CR of ET in cattle. The corpus luteum (CL) diameter (Gonella-Diaza et al., 2013) and the presence of the first-wave dominant follicle (W1DF) ≥ 10 mm (Nishigai, 2003) at the time of transfer affects CR, which indicates that the ovarian state at the time of transfer is an important indicator for recipient selection. Furthermore, our previous study demonstrated that the W1DF located ipsilateral to the CL in the ovary was associated with reduced CR through artificial insemination (AI) in both lactating cows and dairy heifers (Miura et al., 2014), suggesting that the location of the CL in relation to the W1DF affects cattle fertility. Therefore, the location of ovarian structures should be considered during ET recipient selection. According to the recently published report, the location of the CL in relation to the W1DF did not affect the CR after ET (Pugliesi et al., 2019). However, the CRs in ET are affected by several factors, other than the ovarian state of the recipient, such as embryo stage, quality, state (frozen or fresh), etc. (Hasler, 2001; Peixoto et al., 2007). These confounding factors, which can affect CR during ET, were not considered in the aforementioned study (Pugliesi et al., 2019), and this may have masked the effects of the location of the CL in relation to the W1DF on CR.

In the present study, we aimed to investigate whether ovarian state can be considered as an indicator for recipient selection, and the effect of the relationship between the CL and the W1DFlocationon the CR in Holstein-Friesian heifer recipients during JB fresh embryo transfer.

MATERIALS AND METHODS

This study was conducted in accordance with the guidelines for the care and use of laboratory animals at Obihiro University of Agriculture and Veterinary Medicine.

The study was carried out in the area of Tokachi-region, which is located in Hokkaido, in northern Japan and has a subpolar climate. All ET recipients were Holstein-Friesian heifers with an average age of about 14.3±1.9 (average ± standard deviation) months old. These recipients were all kept under equal conditions, with a self-producedgrass silage and management system at the Naitai Plateau public ranch in the Tokachi-region. Donor animals were superovulated with 20 AU of FSH administered twice daily in decreasing doses (5, 5, 3, 3, 2, 2).Recipients were prepared by natural estrus or estrous synchronization using hormone drug treatment prior to transfer. Synchronization treatments for recipients were performed witheither a single intramuscularinjection of PGF2α (0.15mg; Dalmazine; d-cloprostenol, Kyoritsuseiyaku, Tokyo, Japan), oran intravaginal progesterone-releasing device (OVAPRON; Kyoritsuseiyaku, Tokyo, Japan) for 8 days and an intramuscularinjection of estradiol (2 mg; OVAHORMON, estradiol benzoate; ASKA Animal Health, Tokyo, Japan) at progesterone device insertion.The recipients received PGF2α intramuscularinjections two days prior to OVAPRON removal. The end of synchronization treatments was at the time of the last FSH injection.After the end of treatments, heifers were observed for signs of estrus twice per day, for two days; estrus was detected based on clinical signsand rectal palpation.At 6-7 days after the onset of estrus (day 0), one day prior to ET, examination of ovarian structures (CL and W1DF diameter) was performed through trans-rectal ultrasonography (HS-101V; FHK, Tokyo, Japan, with 5MHz linear probe). Clinical findings on the vulva or uterus were also considered during ovarian structures assessment.Heifers with apparent uterine contractions or swollen vulvaat the time of the ultrasoundwere excluded from the study. Embryos were then transferred non-surgicallydeep into the uterus horn ipsilateral to the CL at day 7 or 8 after the onset of estrus using a disposable ET catheter (YT gun; YAMANETECH, Nagano, Japan). Pregnancy diagnosis was performed 53 days following ET using ultrasonography. All embryos in this study were non-surgically collected at day 7 (day 0= estrus) from superovulated JB cows inseminated with frozen JB semen (sex-unsorted). All embryos were evaluated and classified based on the coding system of the Inter-

national Embryo Transfer Society (IETS) for developmental stage and for quality.Among them, the quality code 1 (IETS code 1) embryos were classified into the 'excellent' or 'good' categories, the quality code 2 (IETS code 2) embryos were classified into 'fair' categories.Fresh embryos were encapsulated in a straw and immediately non-surgically transferred to recipients.Data from 297 transfers of JB fresh embryos to virgin Holstein-Friesian heifers, performed between July 2019 and October 2019 by a private company (Zen-noh Embryo Transfer Center), were analyzed. The outcomes were summarized using summary statistics and statistically analyzed by multiple logistic regression model. For analyses with a single variable, the mean valuesof continuous variables between the two groups (W1DF ipsilateral to CL vs W1DF contralateral to CL) were compared using t-test. Comparisons between categorical variables were performed using the chi-square test and the Cochran-Armitage trend tests by using data from analysis of variance and contingency table analyses among groups. The dependent variable in the logistic regression model was the conception status. The independent variables were W1DF ipsilateral to CL (No or Yes), CL diameter (≤ 20mm or > 20mm), W1DF diameter (≤ 10mm or > 10mm), synchronization treatment before transfer (Non-treated or Synchronized), technician code (1, 2, 3, or 4), embryonic quality (Excellent, Good, or Fair) and embryonic stage (Morula, Early blastocyst, or Blastocyst). In the analyses, we categorized CL and W1DF diameter on the basis of the population median. Interactions between the two categorical independent variables were included in this model, but insignificant interactions were removed from the final models ($P \geq 0.05$). Values were considered statistically significant if $P < 0.05$. All statistical analyses were performed using SAS version 9.4 (SAS Institute Japan Ltd., Tokyo, Japan).

RESULTS

In this study, the W1DF was confirmed in 268 of 297 heifers (90.2%). Additionally, W1DF was confirmed in the ovary ipsilateral to CL in 137 (51.1%) and contralateral to CL in 131 (48.9%) out of 268 heifers. As for the size of ovarian structures and recipient age, there were no significant differences between the W1DF ipsilateral to CL group and the W1DF contralateral to CL group; the result of this comparison (t-test) is presented in Table 1.Conceptionrates in the category groups of each confounding variable are shown in Table 2. A total of 211 conceptions (78.7%) were confirmed after ET. As concernembryonic quality, there was a significant tendency for the rate of conception to improve as the embryo quality improved. There were no significant differences regarding the other categorical variables. The relationships between W1DF location in relation to the CL on theovaries and the CRwasanalyzed using multivariable logistic regression model with six confounding variables (Table 3). Our analysis showed that the W1DF location in relation to the CL on ovaries had no significant effect on conception status. A significant association was found between the conception status and embryonic quality.

Table 1. Size of ovarian structures and recipients' age in the heifers having W1DF ipsilateral to CL or W1DF contralateral to CL

	W1DF ipsilateral to CL		
	No (n=131)	**Yes (n=137)**	P
CL diameter (mm)	20.7±2.5* (13.0-30.0)**	20.6±2.2 (16.0-25.0)	0,534
W1DF diameter (mm)	10.7±2.3 (5.0-18.0)	10.6±2.2 (5.0-18.0)	0,547
		DF	
Age (month)	14.4±1.8 (11.5-19.1)	14.3±1.9 (11.5-18.4)	0,601

*: Average ± standard deviation
**: Range
P: t-test

Table 2. Conception status in the category groups of each confounding variable

Variable	Category	N	Conception		*P*	*P*-trend
			n	%		
W1DF ipsilateral to CL	No	131	101	77,1	0,523	-
	Yes	137	110	80,3		
CL diameter	≤20mm	152	118	77,6	0,615	-
	>20mm	116	93	80,2		
W1DF diameter	≤10mm	196	152	77,6	0,436	-
	>10mm	72	59	81,9		
Synchronization treatment before transfer	Non-treated (Natural estrus)	165	132	80,0	0,521	-
	Synchronized	103	79	76,7		
Technician code	1	10	9	90,0	0,276	-
	2	14	13	92,9		
	3	100	74	74,0		
	4	144	115	79,9		
Embryo quality	Excellent	149	125	83,9	0,068	0,031
	Good	77	56	72,7		
	Fair	42	30	71,4		
Embryo stage	Morula	46	37	80,4	0,849	0,589
	Early blastocyst	181	143	79,0		
	Blastocyst	41	31	75,6		

N: number of recipients
n: number of conceptions
P: $\chi 2$
P-trend: cochran-armitage trend test
W1DF: 1st wave dominant follicle
CL: corpus luteum

Table 3. Association between conception status after embryo transfer and the locational relationship between the 1st wave dominant follicle (W1DF) and the corpus luteum (CL) on the ovaries (multiple logistic regression model with six confounding variables)

Variable	Category	EV	SEM	*P*	OR	95% CI
Independent variable						
W1DF ipsilateral to CL	No	-0,065	0,154	0,674	0,878	0.480-1.608
	Yes	Ref				
Confounding variable						
CL diameter	≤20mm	-0,078	0,162	0,630	0,856	0.454-1.613
	>20mm	Ref				
W1DF diameter	≤10mm	-0,109	0,192	0,568	0,804	0.379-1.703
	>10mm	Ref				
Synchronization treatment before transfer	Non-treated (Natural estrus)	0,089	0,164	0,587	1,195	0.629-2.270
	Synchronized	Ref				
Technician code	1	0,448	0,853	0,600	2,266	0.266-19.331
	2	0,732	0,837	0,382	3,011	0.369-24.580
	3	-0,810	0,418	0,053	0,644	0.345-1.204
	4	Ref				
Embryonic quality	Excellent	0,555	0,218	0,011	2,400	1.037-5.558
	Good	-0,234	0,224	0,296	1,091	0.464-2.569
	Fair	Ref				
Embryonic stage	Morula	0,144	0,296	0,626	1,491	0.508-4.377
	Early blastocyst	0,111	0,225	0,623	1,441	0.612-3.391
	Blastocyst	Ref				

EV: estimated value
SEM: standard error of means
P: probability of the reference category in the variable
OR: odds ratio
95% CI: 95% confidence intervals

DISCUSSION

In the present study, the effects of the location of the CL in relation to the W1DF on CR after ET were examined using multivariable logistic regression analysis, which included confounding variables that can affect CR. Miura et al (2014) reported that the development of the W1DF in the ovary ipsilateral to the CL was associated with reduced CR during AI. However, the results from the present study show that the W1DF located ipsilateral to the CL in the ovary did not affect CR after ET. Different results between AI and ET may be related to differences in the environment for early embryo development. Unlike AI, the embryo transferred into uterus is not affected by the oviductal environment. The functions of the oviduct and uterus, which regulate the environment for early embryo development, are controlled by steroid hormones secreted from the ovaries (Chen et al., 2013; Lonergan, 2011). Wijayagunawardane et al (1998) reported that the highest estradiol or progesterone concentration was observed in the oviduct ipsilateral to the pre-ovulatory dominant follicleor to the functional CL, respectively. These resultssuggested that the oviduct environment differs depending on the location of ovarian structures such as W1DF.Therefore, the oviductal environment affected by the W1DF may have an effect on the CR during AI. The present results show that the locational relationships between the W1DF and the CL seems to have no effect on the uterine horns' environment.

It should be noted that only high-quality fresh embryos (characterized as fair, good or excellent) were transferred in this study. After AI, the quality of embryos that reach the uterus varies depending on the quality of the ovum, the state of fertilization, and the environment of the oviduct, which may affect embryo quality.Therefore, in the previous study (Miura et al., 2014) differences in the uterine environment related with differences in the location of ovarian structures may have affected low-quality embryos produced after AI and, consequently, CR.Since, only high-quality embryos are transferred in this study, the effect of the differences in uterine environment on conception rate may be have been masked.In order to clarify this, it may be necessary to include the results of ET using low-grade embryos, such as embryos graded as IETS code-3, in future studies. In addition, this study has shown that the differences in the size of W1DF and CL have no effect on CR. Gonella-Diaza et al. (2013) concluded that CL diameter is an important factor affecting pregnancy ratesafter transfer of in vitro produced embryos (CR 31.4%, after 17, 521 ETs). In our study, CR was very high (78.7%) because we used in vivo produced embryos and this differenceof embryo produced process might account for the conflicting findings. Nishigai (2003) reported that a tendency towards decrease in the CR was observed in recipients having poorly developed CL, high ratio of estradiol-17β toprogesterone in blood plasma, and the coexistence of a follicle ≧10mm in diameter with a CL; however, this decrease was not observed in the cases of good CL development. The present study suggests that the W1DF size does not affect CR under the presence of a functional CL. Our results supported the results of Pugliesi et al (2019) who reported that locational relationship of W1DF and CL has no effects on conception rate. However, there are some differences between their study and ours, recipient's parity, breed or embryo type (in vitro vs in vivo). In the future, we could include more cases, and confounding factors such as recipient's parity, recipient's breed and embryo type to make the analysis model more realistic.

CONCLUSION

In summary, our results showed that the CL and the W1DF diameter and the locational relationship between W1DF and CL on the ovaries did not affect CR after the transfer of fresh high quality JB embryos to Holstein heifers. This suggests that if the recipient heifer has a functional CL, it is not necessary to consider the size and location of the W1DF on the ovaries during recipient selection for the transfer of fresh embryos. This study provides useful information in increasing the efficiency of recipient selection during ET.

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CONFLICT OF INTEREST

None declared.

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