

Relationship between Properties of Cervical Mucus and Pregnancy Rate after Embryo Transfer in Recipient Heifers and Cows

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Abstract: We investigated the relationship between the pregnancy rate of bovine embryo transfer and collapsed protoplasm of epithelial cells in cervical mucus samples collected from 102 cows (Japanese Black, Holstein and their crossbred). The recipient cows were divided into 3 groups according to the proportion of collapsed protoplasm: group L, 20% or less; group M, 21 to 80%; and group H, 81% or more. Rates of pregnancy in these 3 groups were compared. The observation rates of collapsed protoplasm in cervical mucus were 28.4% (29 cows) in group L, 44.1% (45 cows) in group M and 27.5% (28 cows) in group H. The pregnancy rates following embryo transfer were 66.7% (14/21 cows) in group L, 51.4% (18/35 cows) in group M, and 33.3% (8/24 cows) in group H after eighty frozen embryo transfers. The group L and group M recipients had higher pregnancy rates after frozen embryo transfer than group H ($P < 0.05$). The rate of pregnancy increased as the proportion of collapsed protoplasm decreased. There was no significant correlation between pH of mucus, proportion of collapsed protoplasm of epithelial cells and concentration of progesterone in plasma. The present results indicate that there is a relationship between rate of pregnancy and proportion of collapsed protoplasm in the cervical mucus. The high rate of pregnancy in cows with the low proportion of collapsed protoplasm in cervical mucus suggests that the proportion of collapsed protoplasm in cervical mucus is a

reliable index for selection of cows for embryo transfer.

Key words: Embryo transfer, Cervical mucus, Collapsed protoplasm, Selection of recipients

Reported factors affecting the pregnancy rate of embryo transfer in cattle are quality of the embryo [1], method for freezing embryos [2, 3], properties of the blood of recipient cows [4], signs of estrus [5], concentrations of hormones in blood [6–9] and timing of embryo transfer [10]. It is very important to judge correctly whether the embryo should be transferred to recipient cattle, but this decision is usually based simply on the experience of technicians who observe the appearance of the vulva and shape of the corpus luteum. Various methods have been developed in recent years for determining concentrations of hormones and conditions of the corpora lutea in ovaries of recipient cows for embryo transfer. These include a simple method for determining progesterone (P_4) concentration using an enzyme immunoassay technique [11, 12] and a method for determining the shape of the ovaries using an ultrasonic diagnostic apparatus. However, these methods are not easy to perform in the field, and simple, reliable methods are therefore needed.

In 2001, Sasaki *et al.* [13] reported that there was a correlation between pregnancy rate and the ratio of epithelial cells with collapsed muscles in the cervical mucus in the present paper.

In this study, we investigated correlations between changes in epithelial cells in the cervical mucus, pH of

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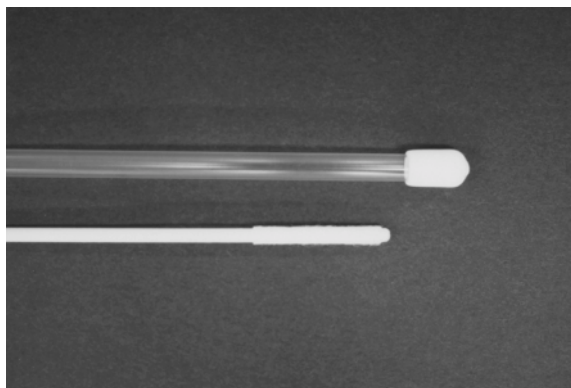


Fig. 1. A cervical mucus sampling device.

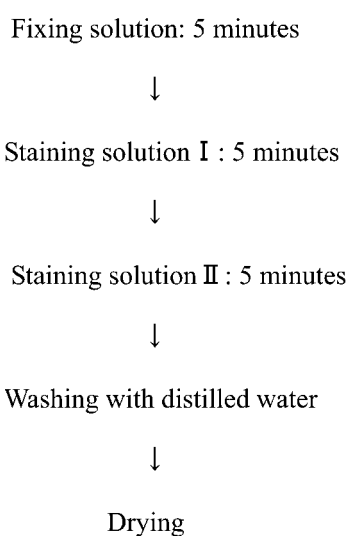


Table 1. Staining method.

cervical mucus, and P₄ concentration in plasma obtained from cows in which embryo transfer was performed.

Materials and Methods

Embryos

Transferrable embryos were collected non-surgically from Japanese Black cows and Holstein cows at the Fukushima Husbandry Experimental Station and private farms in Fukushima Prefecture 7 or 8 days after the onset of estrus. Fresh embryos were transferred soon after collection. The rest of the embryos were frozen by the one-step method equilibrated in phosphate-buffered saline (PBS) supplemented with 10% glycerol. They were thawed in water at 38°C for step-wise removal of

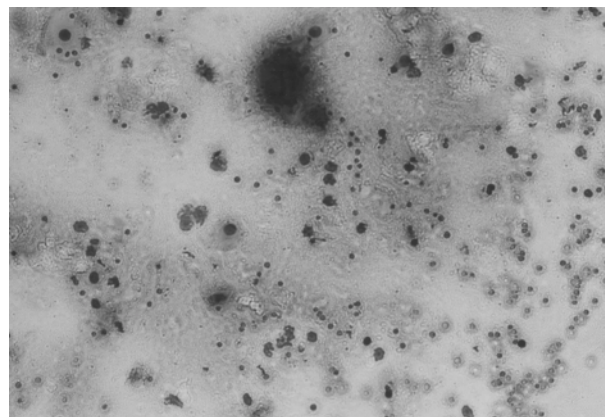


Fig. 2. Epithelial cells with collapsed protoplasm in the cervical mucus (400×).

glycerol with 6% glycerol in 0.25 M sucrose solution, 3% glycerol in 0.25 M sucrose solution, and 0% glycerol in 0.25 M sucrose solution. The embryos were then washed in PBS and transferred to recipient cattle.

Recipients and method for collecting cervical mucus and measuring pH

A total of 102 cattle (Japanese Black, Holstein and crossbred) were used as recipients. All were examined by rectal palpation for the presence of corpora lutea.

A cervical mucus-sampling device was made by wrapping 2 layers of adhesive tape around the tip of a paper stick (50 cm in length) (Fig. 1) to hold a sterilized cotton swab.

For measurement of pH, this device was fitted into a sheath tube cover for embryo transfer, and this device was inserted into the vagina of each cow until it reached the ostium uteri externum. The swab was then slowly pushed into the cervix and then retracted into the sheath tube cover. The device was then removed from the vagina, and the pH of the fluid on the swab was immediately measured using BTB test paper (Advantec-Toyo Corp., Tokyo, Japan).

Samples of cervical mucus collected from the recipient cows were placed on glass slides and stained with staining solution (Diff-Quick, International Reagents Corp., Kobe, Japan). The staining process is shown in Table 1. Samples were assessed under a microscope at a magnification of 400× (Fig. 2).

Blood sampling and determination of plasma progesterone level in recipient cows

Blood samples were collected from the tail vein at the

Table 2. Relationship between pregnancy rate and properties of cervical mucus after transfer of fresh or frozen embryos

Group	Fresh embryo		Frozen embryo	
	Heifer	1≤	Heifer	1≤
L	4/5 (80.0)	0/3 (0)	3/4 (75.0)	11/17 (64.7)
M	3/5 (60.0)	2/5 (40.0)	4/7 (57.0)	14/28 (50.0)
H	0/1 (0)	0/3 (0)	2/4 (50.0)	6/20 (30.0)
Subtotal	7/11 (63.6)	2/11 (18.2)	9/15 (60.0)	31/65 (46.7)
Total	9/22 (40.9)		40/80 (50.0)	

(): Pregnancy rate (%).

same time as cervical mucus collection. Within 1 hour after collection, each blood sample was centrifuged ($1,580 \times g$, 15 min), and the resulting plasma was stored at -20°C until hormone assays. The concentration of progesterone in plasma was measured using an enzyme immunoassay, according to the methods described by Tanaka *et al.* [11] and Takeuchi *et al.* [12].

Statistical analysis

Determination of pregnancy was made at the time of delivery, and data such as pregnancy rate was statistically analyzed using the chi-square test.

Results

Observation of epithelial cells in cervical mucus of the recipient cows

After microscopically observing 30 visual fields, the recipient cows were divided into 3 groups according to differences in the proportion of epithelial cells with a collapsed protoplasm (Fig. 2) in the cervical mucus: group L, 20% or less; group M, 21 to 80%; and group H, 81% or more. The observation rates of collapsed protoplasm in epithelial cells in cervical mucus were 28.4% (29 cows) in group L, 44.1% (45 cows) in group M and 27.5% (28 cows) in group H. There was no statistically significant difference between heifer and cows.

The pH of cervical mucus

The values of pH of cervical mucus from the recipient cows ranged from 6.4 to 7.8. There was no statistically significant difference between heifers and cows, and there were no significant differences among the three groups.

Pregnancy rate after embryo transfer

The relationship between properties of cervical mucus

and pregnancy rate after embryo transfer is shown in Table 2. In this study, the pregnancy rate was not statistically significant different between recipients of fresh embryo transfer and recipients of frozen embryo transfer. Analysis was restricted to frozen embryo transfers because the number of fresh embryo transfers was limited. The group L and group M recipients had higher pregnancy rates after frozen embryo transfer than the group H ($P < 0.05$).

Relationships between proportion of collapsed protoplasm and pregnancy rate in cows grouped according to plasma P_4 concentration

Table 3 shows the pregnancy rate of each protoplasmic disintegration group of cows grouped according to plasma P_4 concentration. Three (3.8%) of the 80 cows had a plasma P_4 concentration of less than 0.9 ng/ml at the time of embryo transfer. The pregnancy rate of these 3 cows was zero. In group L, in which the ratio of presence of images of collapsed protoplasm in the cervical mucus of the womb was low, there was no recipient cow with a P_4 concentration of 2 ng/dl or lower in the blood. There was no correlation between the ratio of pregnancy and the progesterone concentration level. In group H, progesterone concentration levels did not affect the ratio of pregnancy rate. There was no relation between the concentration level of progesterone and the pregnancy rate after embryo transfer.

Discussion

In this study, we examined the mucus in the cervical canal in cows in the luteal phase. We divided these cows into 3 groups according to the proportion of collapsed protoplasm in epithelial cells in the cervical mucus and assessed the correlation between pregnancy rate and the proportion of collapsed

Table 3. Relationships between proportion of protoplasmic disintegration and pregnancy rate in cows grouped according to plasma progesterone concentration

Concentration of progesterone	Proportion of protoplasmic disintegration			Total
	L	M	H	
0.9 ≥	–	–	0/3 (0)	0/3 (0)
1.0~2.0	–	3/6 (50.0)	1/4 (25.0)	4/10 (40.0)
2.1~5.0	11/15 (73.3)	10/21 (47.6)	4/10 (40.0)	25/46 (54.3)
5.1 ≤	3/6 (50.0)	5/8 (62.5)	3/7 (42.9)	11/21 (52.4)
Subtotal	14/21 (66.7)	18/35 (51.4)	8/21 (38.1)	40/77 (51.9)
Total No.	14/21 (66.7) ^a	18/35 (51.4) ^a	8/24 (33.3) ^b	40/80 (50.0)

Values with different superscripts are significantly different ($P < 0.05$). (): Pregnancy rate (%).

protoplasm. There have been few studies on relationships between pregnancy rate and properties of the cervical mucus in the luteal phase [13]. Significant changes occur in the reproductive organs of cows with progression of the estrous cycle, and it has been shown that the properties and amount of cervical mucus are greatly affected by hormones secreted from the ovaries [14]. In the follicular phase, the amount of mucus increases due to the influence of estrogen (E_2), and the viscosity of the mucus decreases, resulting in the formation of a colorless, transparent mucus. If this mucus is dried, fern-shaped crystals are formed. In contrast, the viscosity of the mucus increases in the luteal phase, resulting in the formation of a opaque mucus [14].

As for the origin of the phenomenon of nuclear disintegration that we observed in the present study, Yoshizawa *et al.* [15] examined oviduct cells, and found that most of the ciliated cells in the proliferative phase are large, whereas most of the ciliated cells in the secretion phase (when P_4 concentration is high) are small. The mechanisms responsible for this phenomenon are not clear.

P_4 is produced mainly by cells in the corpus luteum and is involved in the establishment and maintenance of pregnancy. It is thought that a P_4 concentration of more than 1 ng/ml in the blood is generally necessary for the establishment of pregnancy. Nishigai *et al.* [8] reported that the pregnancy rate of recipient cows undergoing embryo transfer decreased as the blood P_4/E_2 ratio on the day before embryo transfer increased. In the present study, none of the cows with a P_4 concentration of less than 0.9 ng/ml at the time of embryo transfer became pregnant, and we believe that this is because the concentration of P_4 in the blood was insufficient. Similarly, Sasaki *et al.* [13] reported that the pregnancy rate in Holstein cows following embryo transfer was

higher in cows with comparatively lower rates of nuclear disintegration in epithelial cells in the cervical mucus. They pointed out that P_4 also affects epithelial cells in the cervical mucus; i.e., when the P_4 concentration decreases, the nuclei of epithelial cells swell slightly and there is a high rate of nuclear disintegration whereas, when the P_4 concentration increases, the nuclei of epithelial cells shrink slightly and there is little disintegration of nuclei (even of nuclei that contain no protoplasm).

The results of the present study show that there is a correlation between pregnancy rate and the proportion of collapsed protoplasm in epithelial cells in the cervical mucus, and that the proportion of collapsed protoplasm could be used as a criterion for selection of cows for embryo transfer.

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References

- 1) Lindner, G.M. and Wright Jr. R.M. (1983): Bovine embryo morphology and evaluation. *Theriogenology*, 20, 407–416.
- 2) Voelkel, S.A. and Hu, Y.X. (1992): Direct transfer of frozen-thawed bovine embryo. *Theriogenology*, 37, 23–37.
- 3) Dochi, O., Imai, K. and Takamura, H. (1995): Birth of calves after direct transfer of thawed bovine embryo stored frozen in ethylene glycol. *Anim. Reprod. Sci.*, 38, 179–185.
- 4) Park, S.B., Im, S.K., Woo, J.S., Kim, I.H., Choi, S.H., Suh, G.H., Ryu, I.S. and Son, D.S. (2000): The usefulness of

plasma urea nitrogen test as an indicator for recipient selection for bovine embryo transfer. *Theriogenology*, 53, 315.

- 5) Tanaka, Y. (1994): Factors of improvement for successful yield rates of recipients for bovine embryo transplantation. *Jpn. J. Embryo Transfer*, 1, 113–119.
- 6) Sunagawa, M., Kasahara, T., Tsunoda, R. and Ohtsu, S. (1987): Morphological investigation of corpus luteum and plasma progesterone levels in bovine recipients. *Jpn. J. Anim. Reprod. Dev.*, 44, 413–419.
- 7) Nishigai, M., Kamomae, H., Tanaka, T. and Kaneda, Y. (1998): Pregnancy rate and blood progesterone concentration on the previous day and the day of frozen embryo of Japanese black. *J. Reprod. Dev.*, 44, 413–419.
- 8) Nishigai, M., Kamomae, H., Tanaka, T. and Kaneda, Y. (2000): The relationship of blood progesterone and estrogen concentration on the day before and the day of frozen-thawed embryo transfer to pregnancy rate in Japanese black beef cattle. *J. Reprod. Dev.*, 46, 235–243.
- 9) Saito, Y. (1991): Clinical findings on endocrine condition for selection of embryo-transfer recipient. *Jpn. J. Anim. Reprod.*, 37, 71P–77P (In Japanese).
- 10) Trounson, A.O., Rowson, L.E. and Willadsen, S.M. (1978): Non-surgical transfer of bovine embryos. *Vet. Rec.*, 102, 74–75.
- 11) Tanaka, S., Nakao, T., Kawahara, T., Moriyoshi, M. and Kawada, K. (1988): A plasma progesterone enzyme immunoassay kit used for heifers. *J. Jpn. Vet. Med. Assoc.*, 41, 83–97 (In Japanese).
- 12) Takeuchi, K., Nakao, T., Moriyoshi, M. and Kawada, K. (1987): Clinical evaluation of progesterone enzyme immunoassay kit for cow's milk. *J. Jpn. Vet. Med. Assoc.*, 40, 95–99.
- 13) Sasaki, K., Kawai, T., Kobayashi, S. and Maeda, J. (2001): Relationship among the properties of cervical mucus, the concentration of plasma level and pregnancy rate in recipient heifer and cows. *J. Anim. Sci.*, 2, j28–j33 (In Japanese).
- 14) Mori, J. (1955): Oestrous of cycle. In: *The textbook of Theriogenology*. (Mori, J., Kanagawa, H., Hamano, K., eds.), pp. 68–69, Bun-Eido Press, Tokyo.
- 15) Yoshizawa, S. (1977): Studies on the cytology of the human fallopian tubes and its clinical application. *RMB*, 22, 20–31 (In Japanese).