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—Research Note—

Preimplantation Development of Embryos in Labrador Retrievers

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Abstract. Preimplantation development of canine embryos is not well understood. To understand the timing of preattachment embryogenesis relative to the luteinizing hormone (LH) surge, early embryonic development was examined in Labrador Retrievers after artificial insemination. The embryos migrated from the oviduct to the uterus beginning on day 11 after the LH surge. This transport must be completed within 24 h. By day 13 after the LH surge, all of the embryos had moved and were localized in the uterus. The embryos developed to the morula stage within 11–13 days and to the blastocyst stage within 14 days after the LH surge, respectively. These findings add to the current understanding concerning the physiology of preimplantation development and should help further develop assisted reproductive techniques in canine species, such as cryopreservation and subsequent embryo transfer.

Key words: Dog, Embryo, Preimplantation development

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The dog is a monoestral polyovulatory nonseasonal species. Canine reproductive physiology is considerably different from other mammalian species. The plasma progesterone concentration of the dog begins to increase a few days before ovulation. Preovulatory luteinization is typical in dogs. The oocytes of dogs are ovulated at the germinal vesicle stage (an immature diploid stage), and they complete meiotic maturation in the oviduct. Thus, canine oocytes and embryos spend a long time prior to implantation in the reproductive tract. Due to these singular reproductive features, the actual situation and mechanisms of early development, such as oocyte maturation, fertilization and subsequent embryogenesis, have not been fully elucidated for canine species compared with many other domestic mammalian species [1].

On the other hand, large numbers of canines are produced and used as working dogs as well as companion animals in many parts of the world. It seems likely that application of assisted reproductive techniques, such as *in vitro* fertilization, embryo transfer, artificial insemination and cryopreservation of gametes and zygotes, would be useful for improved breeding of working dogs, such as guide dogs for the blind, as has proven to be the case in other domestic animals. However, development of assisted reproductive techniques for canines has been poor, with the exception of artificial insemination [2]. In order to develop methods of transfer and cryopreservation of embryos, it is essential to understand early embryonic development *in vivo* in all mammals. However, very little information has been reported regarding the preimplantation development of embryos after mating in dogs. It is believed that fertilized eggs develop to the 2-cell stage 6–10 days after the luteinizing hormone (LH) surge and that they migrate to the uterus 11–12 days after the LH surge in domestic dogs [3, 4]. However, the timing of preattachment embryogenesis relative to ovulation has yet to be determined [5]. Thus, to obtain information regarding early embryonic development in the Labrador Retriever, one of the most utilized working dog breeds in such roles as guide dogs for the blind, embryos were recovered from excised reproductive tracts after artificial insemination, and then the stages and localizations of the embryos were determined.

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Materials and Methods

Both male and female Labrador Retrievers from a breeding colony for guide dogs for the blind were used in the present study. The animals were basically maintained by volunteers in their homes and were moved to the Hokkaido Guide Dog Association when the bitches exhibited signs of estrus. The embryo donors were 13 nulliparous animals that were 9–18 months of age and one multiparous animal that was 8 years of age (Table 1). The plasma progesterone concentrations of the bitches were measured daily by enzyme-linked fluorescent assay (SV-5010; Spotchem Vidas, Arkray, Kyoto, Japan) after the appearance of blood-tinged vaginal discharge. The day the plasma progesterone concentration exceeded 2 ng/ml was considered the day of the LH surge (day 0) [2]. A total of 14 bitches were inseminated by injecting freshly ejaculated semen into the vagina on day 4–6 after the estimated LH surge. The semen was collected from the dogs by digital manipulation. On day 9–15 after the LH surge (day 4–10 after insemination), the reproductive tracts of the bitches were excised after ovariohysterectomy at private clinics. The embryos were flushed from the oviduct, isthmus and uterus with phosphate buffered solution. The developmental stages of the collected embryos were determined under an inverted microscope. The number of ovulated oocytes was estimated by counting the number of corpora lutea present in both ovaries.

The animals used in this study were treated and cared for under the Guiding Principles for the Care and Use of Research Animals

Table 1. Developmental stages of embryos in Labrador Retrievers after artificial insemination

Bitch	Age (M)	Multipara or nullipara	Days post LH surge	Days at AI post LH surge	Days after AI	No. (%) embryos collected/ no. CL	Developmental stage							
							UF	4C	8C	16C	M	ExB	LEB	F
WIN	16	N	9	5	4	5/8 (63)	0	0	5	0	0	0	0	0
ROS	18	N	10	6	4	7/7 (100)	1	0	6	0	0	0	0	0
KOK	16	N	10	4	6	5/8 (63)	1	0	0	4	0	0	0	0
KOH	17	N	11	5	6	7/7 (100)	0	3	1	0	1	0	0	2
HIN	14	N	11	4	7	4/5 (80)	0	0	0	4	0	0	0	0
HAR	10	N	11	4	7	7/8 (88)	0	0	0	6	1	0	0	0
ELU	10	N	12	6	6	9/9 (100)	0	0	0	8	1	0	0	0
EMI	9	N	12	5	7	4/7 (57)	0	0	0	4	0	0	0	0
PET	13	N	13	4	9	4/5 (80)	0	0	0	0	4	0	0	0
PEZ	11	N	14	6	8	8/8 (100)	0	0	0	3	1	0	0	4
DYI	10	N	14	5	9	1/7 (14)	0	0	0	0	0	0	0	1
BET	107	M	14	4	10	5/5 (100)	0	0	0	0	0	5	0	0
JIN	15	N	14	4	10	7/7 (100)	0	0	0	0	0	0	7	0
BIA	9	N	15	5	10	7/10 (70)	0	0	0	0	0	0	7	0
Total			–	–	–	80/101 (79)	2	3	12	29	8	5	14	7

Embryos were collected surgically after non-surgical flushing of the reproductive tract. UF: Unfertilized egg. 4C: 4-cell stage. 8C: 8-cell stage. 16C: 16-cell stage. M: Morula. ExB: Expanded blastocyst. LEB: Large expanded blastocyst. F: Fragmented.

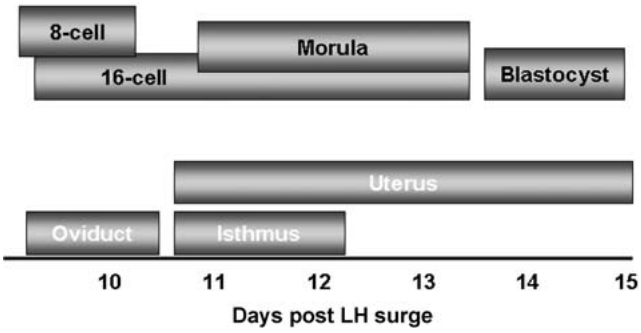

Fig. 1. Preimplantation development and localization of embryos in the Labrador Retrievers. The day the plasma concentration of progesterone exceeded 2 ng/ml was considered to be the day of the LH surge (day 0). The embryos developed to the morula and blastocyst stages by 11–13 and 14 days after the LH surge, respectively. The embryos began to migrate from the oviduct to the uterus on day 11 after the LH surge. By day 13 after the LH surge, all of the embryos had moved and were localized in the uterus.

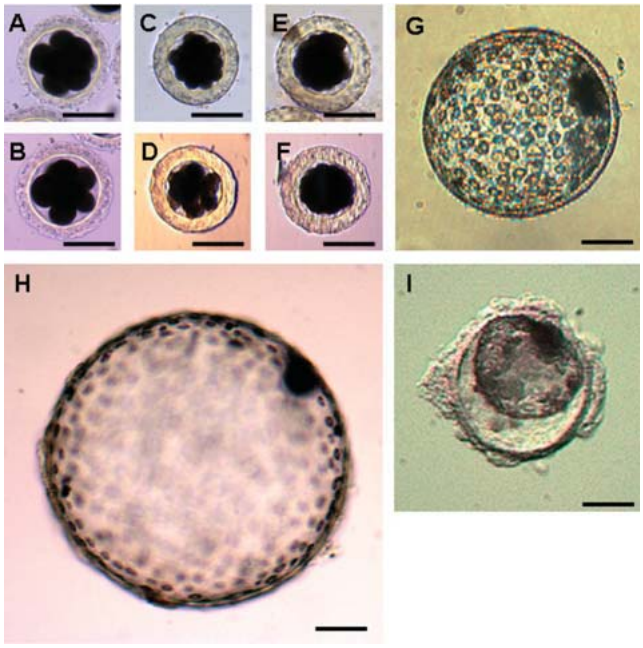

Fig. 2. Preimplantation stages of embryos recovered from the female reproductive tract after artificial insemination in the Labrador Retriever. A, B: 8-cell stage. C, D: 16-cell stage. E, F: Morula. G: Expanded blastocyst. H: Large expanded blastocyst. I: Shrunken blastocyst. Bars indicate 100 μm.

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Results and Discussion

To be able to utilize assisted reproductive technologies in research and clinical practice, it is essential to be able to determine the timing of ovulation. Although the onset of vulvar bleeding, vaginal smears and mating behavior have been used to assess the timing of ovulation, their relative accuracy has proven to be highly variable. However, ovulation is much more closely related to the LH surge and progesterone concentration. There is little variation in the 2-day interval between the preovulatory LH surge and ovulation (36–50 h) [1]. The blood progesterone concentration at ovulation is also relatively stable and is thus extensively used to estimate ovulation time [1]. Since measurement of the blood LH concentration is difficult to routinely carry out (i.e., it is time-consuming), we utilized measurement of the progesterone

Table 2. Localization of embryos in the reproductive tract of Labrador Retrievers after artificial insemination

Bitch	Days post LH surge	Days after AI	No. embryos collected	Localization of embryo		
				Oviduct	Isthmus	Uterus
WIN	9	4	5	5	0	0
ROS	10	4	7	7	0	0
KOK	10	6	5	5	0	0
KOH	11	6	7	0	7	0
HIN	11	7	4	0	3	1
HAR	11	7	7	2	1	4
ELU	12	6	9	0	0	9
EMI	12	7	4	0	0	4
PET	13	9	4	0	0	4
PEZ	14	8	8	0	0	8
DYI	14	9	1	0	0	1
BET	14	10	5	0	0	5
JIN	14	10	7	0	0	7
BIA	15	10	7	0	0	7
Total	–	–	80	19	11	50

Embryos were collected surgically after non-surgical flushing of the reproductive tract.

concentration to predict the LH surge in the present study.

Table 1 shows the developmental stages of the embryos from the Labrador Retrievers after artificial insemination with freshly collected semen. A total of 80 embryos were collected from 14 bitches that possessed a total of 101 corpus lutea (recovery rate: 79.2%). The recovery rate was 100% in 6 of the 14 animals (44%). The mean ovulation rate of the Labrador Retrievers used in the present study was 7.2 (n=14). The blastocyst stage of the embryos did not appear within 13 days after the LH surge. Beginning 14 days after the LH surge, blastosysts were recovered from the uterus (Fig. 1). In a previous study using Beagles, blastocyst stage embryos were recovered on days 9 and 10 after the LH surge [6]. These results indicate that there is a considerable breed-related difference in the preimplantation development of embryos in canine species. In the present study, a majority of the expanded blastosysts were found to be shrunken at the time of collection or during washing of the embryos (Fig. 2-I). However, shrinkage of the blastocyst stage embryos was not observed during recovery.

Table 2 shows the localization of the embryos in the reproductive tract of the Labrador Retrievers after artificial insemination with freshly collected semen. Embryos that developed into morula migrated from the oviduct to the uterus beginning on day 11 after the LH surge. By day 13 after the LH surge, all of the embryos had moved to and were localized in the uterus. Although the embryonic stages of the collected embryos were synchronized in a majority of the bitches, the fact that the collected embryos from one particular bitch (KOH) exhibited a wide range of developmental stages, from the 4-cell stage to the morula stage, might indicate that ovulation can extend over a long period of time in some animals (Table 1). It is generally believed that ovulation from both ovaries is completed in a maximum of 24 to 36 h [1]. When freshly ejaculated semen was inseminated 4–6 days after the estimated LH surge, morula- and blastocyst-stage embryos (Fig. 2), which appear to be suitable for cryopreseravation and embryo transfer, might be collectable from the uterine horns of Labrador Retrievers on days 13 and 14, respectively (Fig. 1).

In conclusion, as shown in Fig. 1, the present study demonstrates that embryos migrate from the oviduct to the uterus beginning on day 11 after the LH surge in the Labrador Retrievers. This transport might be completed within 24 h. The embryos developed to the morula and to blastocyst stages by 11–13 and 14 days after the LH surge, respectively. Fundamental research is still required to understand the mechanisms of fertilization and early embryonic development in order to better control and mimic them *in vitro* in canines.

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