

Supplementary information

Scheduled simple production method of pseudopregnant female mice for embryo transfer using the luteinizing hormone-releasing hormone agonist

Gema Puspa Sari ¹, Shunsuke Yuri ¹, Arata Honda ², and Ayako Isotani ^{1*}

1 Division of Biological Science, Graduate School of Science and Technology, Nara Institute of Science and Technology, 8916-5 Takayama-cho, Ikoma, Nara, 630-0192, Japan

2 Center for Development of Advanced Medical Technology, School of Medicine, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-shi, Tochigi-ken, 329-0498, Japan

*Corresponding author:

Ayako Isotani

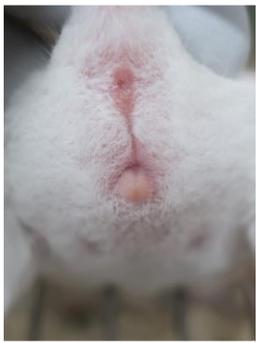
E-mail address isotani@bs.naist.jp

Supplementary Fig. S1

Exp. group	D1	D2	D3	D4	D5	D6
group_4	10 µg	10 µg			pairing	plug check
group_5	20 µg	10 µg			pairing	plug check
group_6	10 µg	20 µg			pairing	plug check
group_7	40 µg				pairing	plug check
group_8		40 µg			pairing	plug check

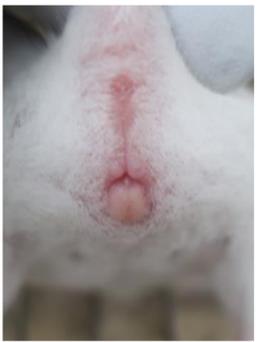
Supplementary Fig. S1

Scheme of LHRHa administration schedules and treated LHRHa concentrations.



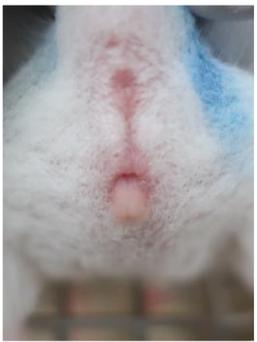
group_3-01

Visual	-
Plug	-



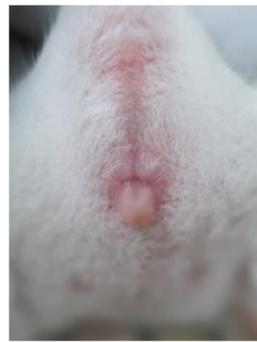
group_3-02

Visual	+
Plug	+



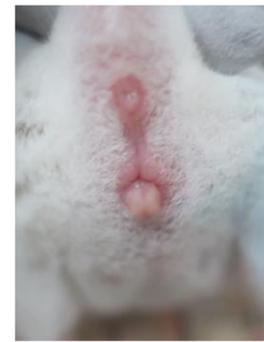
group_3-03

Visual	+
Plug	+



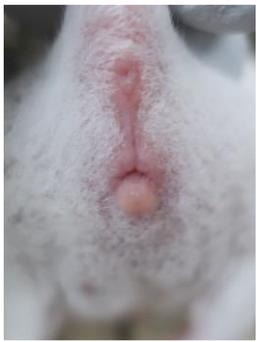
group_3-04

Visual	+
Plug	-



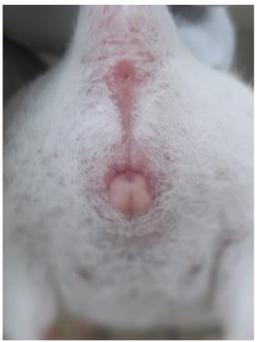
group_3-05

Visual	+
Plug	+



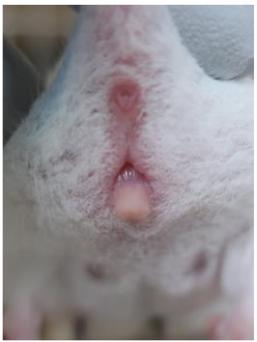
group_3-06

Visual	+
Plug	+



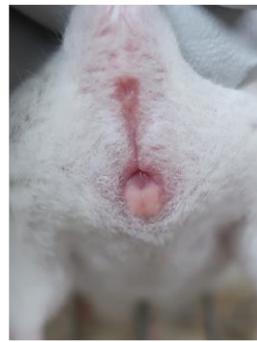
group_3-07

Visual	-
Plug	-



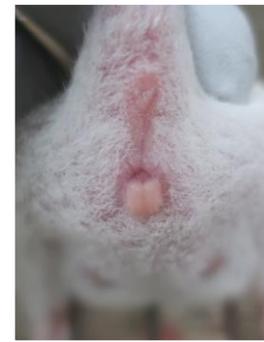
group_3-08

Visual	+
Plug	+



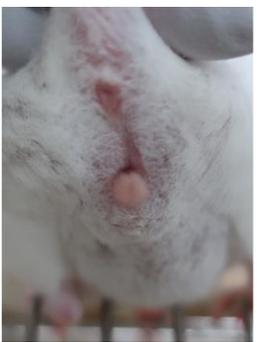
group_3-09

Visual	+
Plug	+



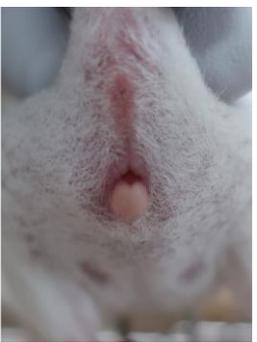
group_3-10

Visual	+
Plug	+



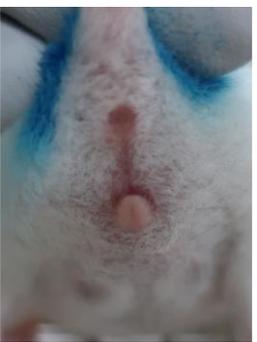
group_3-11

Visual	-
Plug	-



group_3-12

Visual	+
Plug	+



group_3-13

Visual	-
Plug	+



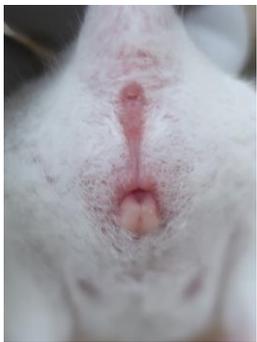
group_3-14

Visual	+
Plug	+



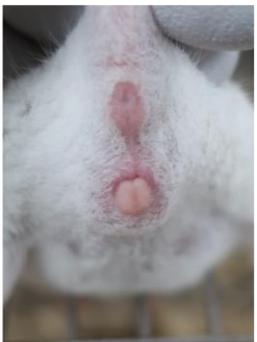
group_3-15

Visual	+
Plug	+



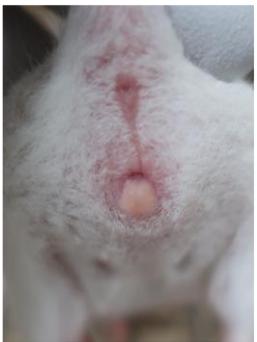
group_3-16

Visual	+
Plug	+



group_3-17

Visual	+
Plug	+



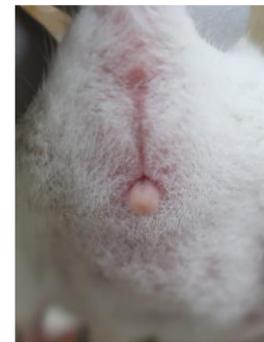
group_3-18

Visual	-
Plug	+



group_3-19

Visual	-
Plug	+



group_3-20

Visual	-
Plug	-

Supplementary Fig. S2 Pictures of mouse vaginas for visual method after the LHRHa administration in the group_3.

Vaginal pictures were taken just before mating with male mice (D5). Each female mouse was administrated LHRHa in the group_3 condition, judged the potency of mating by visual method, and observed a plug the next day (D6). Visual “+” means the female was judged the high potency of mating with a male. Plug “+” means the female had a plug after mating with a male.

Supplementary Table S1

The frequency of copulation on each estrus stage

Estrus stage	total	plug-positive	%
Proestrus	41	26	63
Estrus	178	114	64
Metaestrus	28	2	7
Diestrus	157	21	13

Supplementary Table S2

Effects of LHRHa treatment by several conditions

exp. group	No. (%) of females		Litter size ^(#3)	Viability of offspring (%)	Body weight of offspring (g) ^(#4)
	With plug ^(#2)	Pregnant			
group_4	6/13 (46)	3/3 (100)	13 ± 1	37/39 (95)	1.9 ± 0.1*
group_5	3/8 (38)	2/2 (100)	14 ± 1	27/27 (100)	1.8 ± 0.2
group_6	4/8 (50)	2/2 (100)	22 ± 1	43/43 (100)	1.8 ± 0.2
group_7 ^(#1)	1/5 (20)	0/1 (0)	N/D	N/D	N/D
group_8	8/13 (62)	7/7 (100)	15 ± 2	103/103 (100)	1.8 ± 0.1

(#1) Since a plug-positive female mouse in group_7 did not become pregnant, data of offspring were shown as not determined (indicated as N/D).

(#2) Plug were checked one day after pairing with VAS male or WT mice.

(#3) n: All pregnant females in each experimental group.

(#4) n: All viable offspring in each experimental group.

Fisher's exact probability test was performed for With plug, Pregnant and Viability of offspring, and Bonferroni correction on Kruskal-Wallis test was performed for Litter size and Body weight of offspring. * P < 0.01 vs. the corresponding value of the control in Table 2.