COSMO BIO CO., LTD.

CARD HyperOva (Superovulation Reagent for mouse)

Cat. No.	KYD-010-EX	Size:	1 mL
	KYD-010-EX-X5		5*1 mL

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Origin

Serum of goat, Horse-derived villus gonatropin.

Composition

1. Inhibin antiserum (Goat)
2. Equine chorionic gonatropin.

Protocol

Materials and Equipment:

1. Superovulation reagent (CARD HyperOva; Cat.# KYD-010-EX)
2. hCG (human Chorionic Gonadotropin, CG-10; Sigma) (37.5 IU/mL in sterile saline)
3. 1 mL disposable syringe
4. FERTIUP (Preincubation medium: PM, Cat.# KYD-002-EX)
5. CARD MEDIUM (Cat.# KYD-003-EX)
6. mHTF (HTF (Cat. # CSR-R-B070) can be used same as mHTF.)
7. Micropipettes
8. Pipette tips for preparation of dishes
9. Pipette tips for insemination (Pipette Tip Cat. # 114; Quality Scientific Plastics)
10. Plastic dishes (35 mm X 10 mm Cat. # 430588; CORNING)
11. Fine scissors
12. Pair of watchmaker's #5 forceps
13. Micro-spring scissors (5 mm blade)
14. Dissecting needle
15. Filter paper
16. Glass capillaries for embryo handling
17. Microscope
18. Humidified incubator (37° C, 5% CO_2, 95% air)

Procedure:

Preparation of CARD HyperOva®

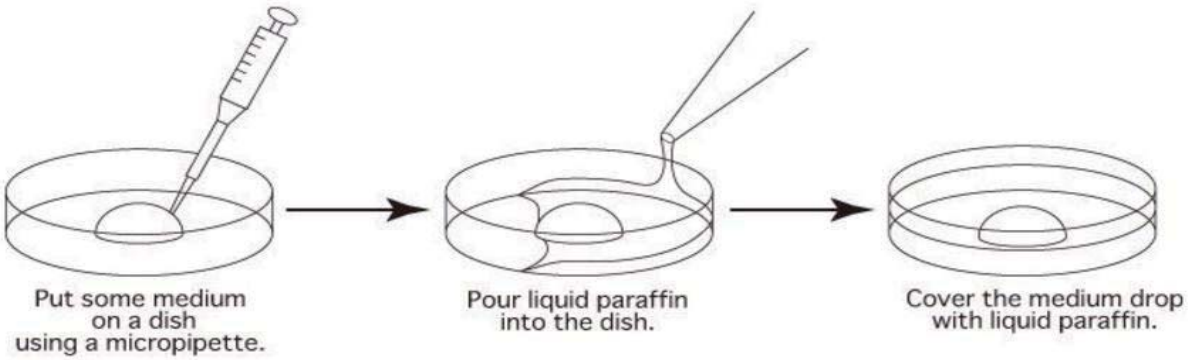
1. Take CARD HyperOva® out from freezer, and thaw it in an incubator or in water bath at 37℃ for 10 minutes. Administer CARD HyperOva® to mouse within 1 hour after thawing.
2. Before administration, invert the tube for several times in order to mix CARD HyperOva® well.
3. There might be some clumps in CARD HyperOva®. Avoid them when aspirating CARD HyperOva® in a sylinge (The clumps don't affect the effectiveness of CARD HyperOva®).

Superovulation

1. Induce superovulation by injecting 0.1-0.2 mL of CARD HyperOva i.p. into a 26-30 days old female mouse (counting the date of birth as day 0). (CARD HyperOva is usually administered during the light cycle, between the hours of 17:00 and 18:00).
2. Follow this up 48 hours later with a 7.5 IU i.p. injection of human chorionic gonadotropin (hCG).

Preparation of Dishes

1. Prepare dishes as per the instructions below and keep them in an incubator (37° C, 5% CO_2 in air) to allow them to gas-equilibrate.



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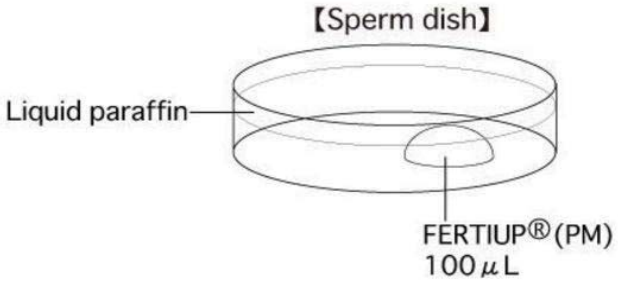
a. Sperm dish

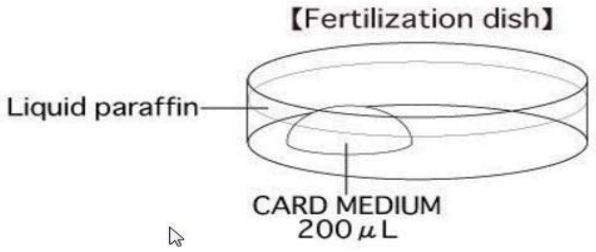
Put 1 drop (100 µL/drop) of FERTIUP (PM) into a dish and cover it with liquid paraffin 30 minutes before collecting sperm, and place the dish in an incubator.



b. Fertilization dish

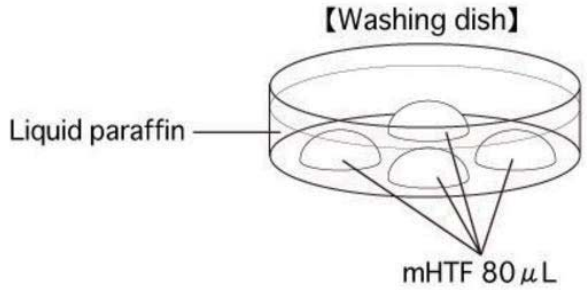
Put 1 drop (200 µL/drop) of CARD MEDIUM into a dish and cover it with liquid paraffin 10 minutes before collecting oocytes, and place the dish in an incubator.

Note: There are three different methods of preparing CARD MEDIUM, depending on whether in vitro fertilization will be carried out using fresh, frozen-thawed or cold-temperature transported spermatozoa.
Please refer to the CARD MEDIUM instruction manual.
http://search.cosmobio.co.jp/cosmo_search_p/search_gate2/docs/KYD_/003EX.20140808.pdf



c. Washing dish

Put 4 drops (80 µL/drop) of mHTF into a dish and cover them with liquid paraffin. Place the dish in an incubator for at least 30 minutes.



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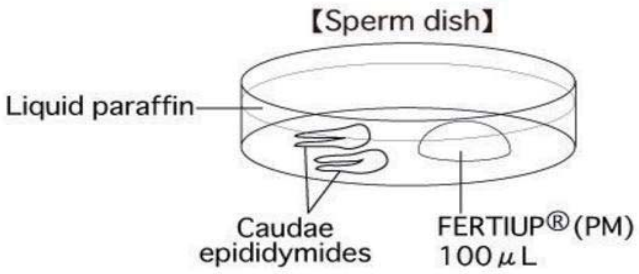
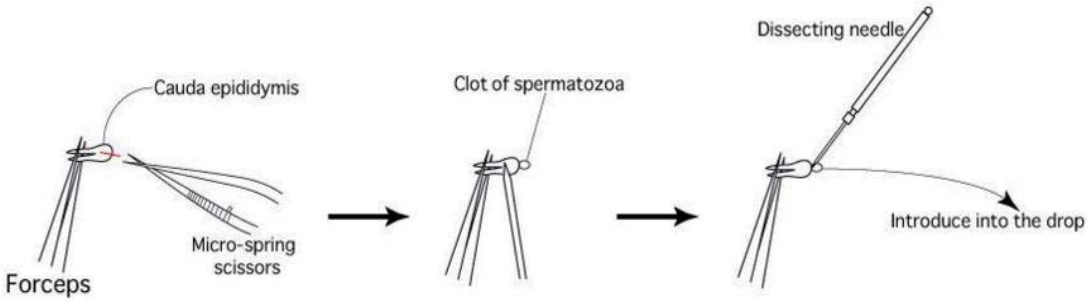
Collection of Spermatozoa

1. Sacrifice 1 or 2 mature male mice (3 to 6 months old) and remove their cauda epididymides, avoiding as much fat, blood and tissue fluid as possible.
2. Place the tissue on sterile filter paper to blot away any blood and fluid.
3. Place the removed cauda epididymides in a sperm dish containing liquid paraffin.



4. Cut the duct of each cauda epididymis using a pair of micro-spring scissors, then use a dissecting needle to gently press the surface of the cauda epididymis and release the sperm within.
5. Use a dissecting needle to introduce the clots of spermatozoa released from the cauda epididymides into the drop of FERTIUP (PM).



Note: The degree of fertility varies greatly depending on the spermatozoa used. Spermatozoa with high fertility levels can be observed moving in a vortex with high motility at the boundary of the incubation medium. Conversely, spermatozoa which display low motility and poor homogeneity tend to have low fertility levels.

6. Allow the sperm to capacitate by placing the suspension in an incubator (37℃ , 5% CO_2 in air) for 60 minutes before insemination.

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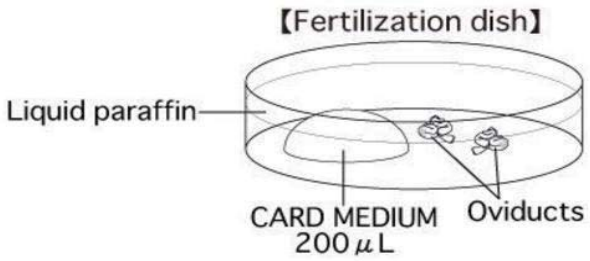
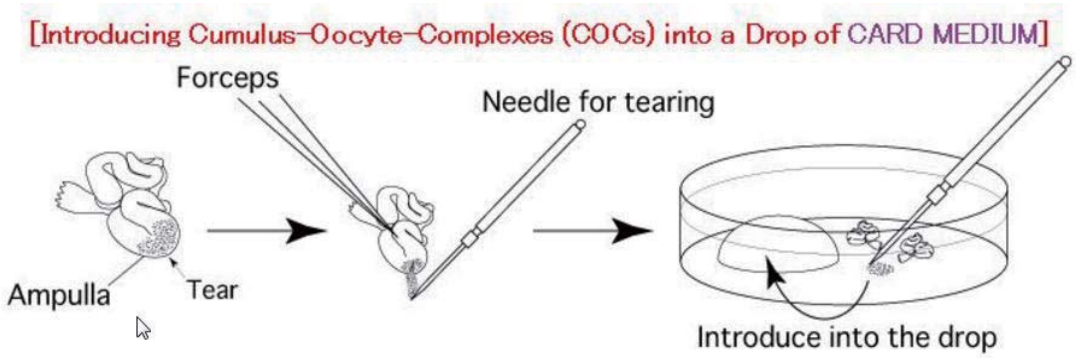
Collection of Oocytes

1. Sacrifice a superovulating mature female mouse (26-30 days old) approximately 15-17 hours after administering hCG.
2. Dissect the mouse to expose the abdominal cavity.
3. Move the digestive tract from inside the abdomen and expose the uteruses, oviducts and ovaries.
4. Remove the uteruses, oviducts and ovaries, and place them on sterile filter paper.
5. Remove the oviducts (ampullae) only, avoiding as much fat, blood and tissue fluid as possible.
6. Immerse the removed oviducts in liquid paraffin contained within a fertilization dish.



7. Use forceps to hold the oviduct against the base of the fertilization dish, then use a dissecting needle to tear open the ampulla of the oviduct and release the cumulus-oocyte-complexes (COCs) from within. Drag them into the drop of CARD MEDIUM (200 µL).



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Note: Be sure to carry out all operations, from sacrificing the female and removing her oviducts to introducing the COCs into a drop of CARD MEDIUM, in the shortest time possible (within 30 seconds).

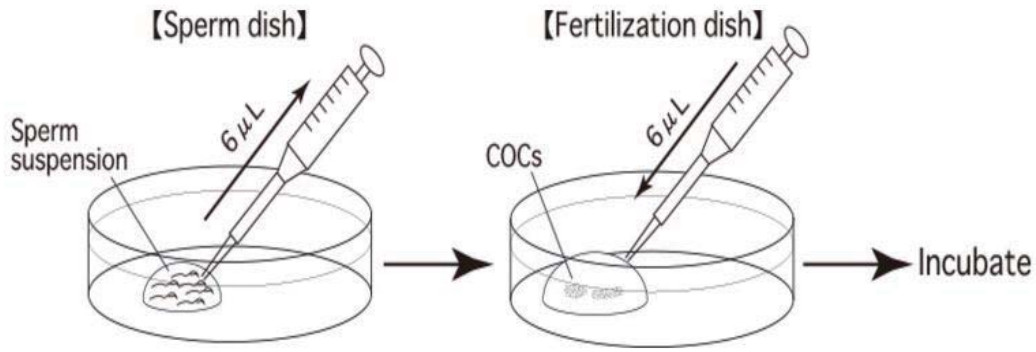
Moreover, when carrying out this process alone, do not sacrifice multiple mice at once; instead, sacrifice one mouse and swiftly remove its oviducts before moving on to the next mouse.

Use one drop of CARD MEDIUM (200 µL) per female (2 oviducts).

8. Keep the fertilization dish including COCs in an incubator (37℃ 5% CO_2 in air) for 30-60 minutes before insemination.

Insemination

1. Use the tip of a pipette (Pipette Tip Cat.No.114; Quality Scientific Plastics) to add appropriate amounts (usually about 6 µL) of the sperm suspension to the drop of CARD MEDIUM containing the COCs.
2. Place the fertilization dish in an incubator (37° C, 5% CO_2 in air).



3. 3hours after insemination, wash the oocytes 3 times in fresh mHTF (80 µL) in a washing dish, avoiding the transfer of CARD MEDIUM.
4. 6 hours after insemination, observe the oocytes in the third drop of mHTF and remove any parthenogenetic oocytes which have only one pronucleus.

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Note: At this stage it is important that you identify and remove any parthenogenetic oocytes. Please note that if you do not remove the parthenogenetic oocytes at this stage, the next day they develop to the 2-cell stage, at which point it will be impossible to distinguish the fertilized oocytes from the parthenogenetic oocytes.

Note: The fertilized oocyte has both a male and female pronucleus (A).
On the other hand, the parthenogenetic oocyte has only one pronucleus (B) and the unfertilized oocyte does not have any pronuclei (C).

5. After overnight culture of the oocytes, transfer the obtained 2-cell stage embryos only to the fourth drop of mHTF in a washing dish. These embryos can be vitrified, transferred to recipient females, or cultured to the blastocyst stage. (Please refer to the chapters of Simple Vitrification of Mouse Embryos and Embryo Transfer into the Oviduct.)

References

- Takeo T., Hoshii T., Kondo Y., Toyodome H., Arima H., Yamamura KI., Irie T., and Nakagata N. 2008. Methyl-beta-cyclodextrin improves fertilizing ability of C57BL/6 mouse sperm after freezing and thawing by facilitating cholesterol efflux from the cells. Biol Reprod. 78(3): 546-51.
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- Takeo T., and Nakagata N. 2011. Reduced glutathione enhances fertility of frozen/thawed C57BL/6 mouse sperm after exposure to methyl-beta-cyclodextrin. Biol Reprod. 85(5):1066-1072.
- Takeo T., Nakagata N. 2015 Superovulation Using the Combined Administration of Inhibin Antiserum and Equine Chorionic Gonadotropin Increases the Number of Ovulated Oocytes in C57BL/6 Female Mice. PLoS One. 2015 May 29;10(5):e0128330. doi: 10.1371/journal.pone.0128330.

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