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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

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Code of practice for in vitro embryo production and transfer in cattle

牛体外胚胎生产和移植技术规程

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1 Scope

This document establishes the operating procedure for the in vitro production and transfer of cattle embryos, specifies the technical requirements for the basic requirements of in vitro embryo production, the selection and treatment of donors, the collection of oocytes, in vitro maturation, in vitro fertilization, in vitro culture, the freezing and thawing of embryos, and embryo transfer and pregnancy diagnosis, and describes the methods of verification.

This document applies to the in vitro production and transfer of cattle embryos.

2 Normative references

The contents of the following documents constitute indispensable provisions of this document through normative reference in the text. For dated references, only the edition corresponding to that date applies to this document; for undated references, the latest edition (including all amendments) applies to this document.

GB/T 6682, Water for analytical laboratory use - Specification and test methods. GB/T 25881, Bovine embryos. GB/T 26938, Code of practice for in vivo embryo production and transfer in cattle.

3 Terms, definitions and abbreviated terms

The terms and definitions given in GB/T 25881 and GB/T 26938 and the following apply to this document.

3.1.1 cumulus-oocyte complexes, COCs: complex formed by an oocyte enclosed in cumulus cells.

3.1.2 in vitro maturation, IVM: process in which COCs are cultured in vitro under suitable conditions so that the oocyte matures, extrudes the first polar body and acquires the capacity to be fertilized.

3.1.3 sperm purification: operation by which live sperm are separated from semen.

3.1.4 in vitro fertilization, IVF: process in which sperm and a matured oocyte complete fertilization in an in vitro environment.

3.1.5 in vitro culture, IVC: process in which a fertilized ovum is cultured in a suitable in vitro environment.

3.2 The following abbreviated terms apply to this document: BLR, blastocyst rate; CLR, cleavage rate; CR, calving rate; FSH, follicle-stimulating hormone; OPU, ovum pick-up; PR, pregnancy rate.

5 Basic requirements

5.1 Facilities and equipment. The following are advised: a) facilities, a clean laboratory of class one million or better; b) instruments and equipment, an ovum pick-up unit, a stereomicroscope, a centrifuge, a sperm density analyser, a carbon dioxide incubator, a three-gas incubator, a heated stage and a water bath.

5.2 Culture conditions. In vitro maturation of oocytes and in vitro fertilization are to be carried out at 38.5 degrees C in air containing 5 % carbon dioxide by volume at saturated humidity; in vitro embryos are to be cultured at 38.5 degrees C in 5 % carbon dioxide, 7 % oxygen and 88 % nitrogen by volume at saturated humidity.

5.3 Reagents. 5.3.1 Chemical reagents are to be of a purity meeting the requirements for cell or embryo culture grade. 5.3.2 Water used in the work is to meet the requirements of GB/T 6682 for grade two water, with a resistivity of 18.2 megohm centimetre at 25 degrees C.

6 Selection and treatment of donors

6.1 Selection of donors. A donor is to meet the following conditions. a) It conforms to the standard for its breed, its genetic performance is stable, its breeding value is high and its pedigree is clear over three generations. b) Its reproductive organs and ovarian function are normal. c) A heifer used for ovum pick-up is advised to be over 10 months of age and a cow that has calved to be more than 40 days past calving; body condition is to be moderate, with a score advisedly above 2.75 on a five-point scale. d) It is free of hereditary defects and of infectious disease.

6.2.1 Treatment without superovulation. Oocytes are collected directly with the ovum pick-up unit, advisedly once every three to four days.

6.2.2 Treatment with superovulation. The superovulation programme for the donor is as follows: a) on day 0 the ovaries are examined with the ovum pick-up unit and follicles more than 5 mm in diameter are advisedly punctured; b) on day 2 and day 3, FSH is injected once every 12 hours, the appropriate dose being set according to the activity of the FSH

preparation and the breed and condition of the donor; c) on day 5 ovum pick-up is carried out; d) the interval between repeated superovulation treatments is advisedly more than 14 days.

7 Collection of oocytes

7.1 Collection of oocytes from ovaries after slaughter of the donor. Fresh ovaries taken after slaughter are placed in physiological saline containing antibiotics at 20 degrees C to 25 degrees C and transported to the laboratory within 6 hours. They are washed three to four times with physiological saline pre-warmed to 30 degrees C to 37 degrees C. A disposable syringe or a vacuum pump is connected to a 21 G needle, the needle is inserted with its opening downwards into follicles 2 mm to 8 mm in diameter, and the follicular fluid and the COCs are aspirated and transferred into a cell culture dish placed on a heated stage at 38.5 degrees C; the COCs are collected under a stereomicroscope and transferred into collection medium (see A.1 in Annex A) pre-warmed to 38.5 degrees C.

7.2.1 Preparation before collection. The donor is restrained, the rectum is emptied of faeces, epidural anaesthesia is given, and the perineum and vulva are washed and disinfected; a 50 mL centrifuge tube is connected to the vacuum pump and the collection needle and placed in a heated bath; a small quantity of collection medium is drawn through to rinse the collection line.

7.2.2 Collection procedure. The procedure is as follows. a) With one hand the operator inserts the ultrasound probe of the ovum pick-up unit into the vagina of the donor as far as the fornix, and with the other hand, working through the rectum, holds one ovary firmly against the probe through the wall of the vaginal fornix. b) The relative position of the probe and the ovary is adjusted so that a follicle lies on the needle line; the vacuum pump is switched on, the collection needle is advanced to puncture the follicle, and the follicular fluid and the COCs are collected. c) When all follicles 2 mm to 8 mm in diameter have been collected, the needle and the line are flushed so that the COCs pass into the centrifuge tube. d) The other ovary is worked in the same way. e) If blood blocks the needle or the line, the needle is withdrawn, the vacuum is raised and collection medium is drawn through to clear the needle and the line. f) The collected follicular fluid and COCs are transferred to a collection cup where the liquid is filtered off; the cup is placed on a heated stage at 38.5 degrees C, the COCs are collected under a stereomicroscope and transferred into collection medium pre-warmed to 38.5 degrees C.

8 In vitro maturation

8.1 Preparation of maturation medium droplets. Droplets of 100 microlitres of maturation medium (see A.2) are made in a cell culture dish, covered with mineral oil and equilibrated in the carbon dioxide incubator for at least 2 hours.