Preparation of Hormones for Superovulation

1.0 Equipment

1.1 Eppendorf and Universal holders

1.2 Safety glasses

1.3 Measuring cylinder

1.4 – 20°C Freezers

1.5 Osmometer

1.6 pH meter

2.0 Supplies

2.1 10ml Gilson pipette with tips

2.2 Sterile media bottle

2.3 21 gauge x 1.5″ needle

2.4 1ml syringe

2.5 20 ml Universals

2.6 1.5ml Eppendorfs

2.7 Brady labels with appropriate information

2.8 NHPP (PMS) supplied as 2,000 I.U

2.9 Prospec (PMS) supplied as 5,000 I.U

2.10 Generon (PMS) supplied as 1,000 I.U

2.11 NHPP (hCG) supplied as 10,000 I.U

2.12 Sigma (hCG) supplied as 2,500 I.U

2.13 Chorulon (hCG) supplied as 1,500 I.U

2.14 DPBS (Sigma, Cat no:14190-094)

2.15 Prospec (hCG) – activity 6,098U/mg in 10mg vials

2.16 Osmometer calibration solutions

2.17 pH Buffer solutions

3.0 Procedure

3.1 General information

3.1.1 **PMSG:** To calculate volumes for different strengths use the following formula: strength of 1 vial of PMS divided by strength required, divided by 10 equals volume of DPBS required per vial.

e.g. For a required strength of 5iu from 1 vial of 5000iu PMS:

5000iu/5iu = 1,000.

1,000/10 = **100ml** DPBS per vial

Prospec (5000iu)

5iu = 1 vial PMS + **100ml** DPBS

NHPP (2000iu)

5iu = 1 vial of PMS + **40ml** DPBS

Generon (1000iu)

5iu = 1 vial of PMS + **20ml** DPBS

3.1.2 **hCG**: To calculate volumes for different strengths use the following formula: strength of 1 vial of hCG divided by strength required, divided by 10 equals volume of DPBS required per vial.

e.g. For a required strength of 5iu from 1 vial of 1,500iu hCG:

1,500iu/5iu = 300

300/10 = **30ml** DPBS per vial

Chorulon (Intervet) (1,500iu)

5iu = 1 vial of hCG + **30ml** DPBS

NHPP (10,000iu)

5iu = 1 vial of hCG + **200ml** DPBS

Sigma (2,500iu)

5iu = 1 vial of hCG + **50ml** DPBS

Prospec (Activity 6,098U/mg)

10mg vial = 60,980iu

5iu: (60,980iu/5iu)/10 = **1,219.6ml** DPBS

1 vial + 10ml DPBS = 10x 1ml aliquots of 6098 units.

Then dilute the 1ml aliquot in **121.96ml** DPBS to give a working solution of 50iu/ml (5iu/100ul)

NOTE: Calculated volumes can be multiplied to accommodate a higher quantity of hormone preparation. (i.e. 5iu hCG (Chorulon) = 7 vials of hCG + **210ml** DPBS).

3.1.3 Discard the vials of diluent (liquid) that are supplied with Chorulon (hCG) in the glass bin.

3.2 To make 5iu PMSG

3.2.1 Measure the required volume of DPBS into a clean measuring cylinder and pour it into a sterile media bottle.

3.2.2 Using a 1ml syringe and a 21 gauge x 1.5″ needle, aspirate 1ml of DPBS from the sterile bottle. Puncture the rubber lid and expel the contents of the

syringe into the vial containing the powdered PMSG hormone (stored in freezer or refrigerator according to brand).

3.2.3 Invert vial 3-4 times to dissolve powder.

3.2.4 Using the same syringe and needle, aspirate the hormone solution and expel it into the sterile bottle containing DPBS.

3.2.5 Aspirate 1ml of the diluted hormone from the sterile bottle and expel it into the empty hormone vial. Invert the vial, then aspirate the solution and expel it into the sterile bottle.

3.2.6 Repeat step 3.2.5 x 3 times per vial. This ensures that as much as possible of the powdered hormone is washed out of the vial.

3.2.7 Repeat steps 3.2.2 - 3.2.6 for the desired number of vials of the powdered hormone required.

3.2.8 Invert the sterile bottle several times to ensure the solution is completely mixed.

3.2.9 Using a P10 Gilson pipette and a 10ml Diamond pipette tip, aspirate 10ml of the hormone solution into a sterile universal tube and label 'PMSG (date) for pH and osmolality testing'.

3.2.10 Aliquot the hormone solution into either;

- 1.4 ml into 1.5ml Eppendorfs (this will treat approx. 8x mice)

  and/or

- 4.6ml into 20ml Universal tubes (this will treat approx. 25x mice).

3.2.11 Apply labels on the Eppendorfs and Universals stating "PMSG", "strength e.g 5iu" and "date".

3.3 To make 5iu hCG

3.3.1 Measure the required volume of DPBS into a clean measuring cylinder and pour it into a sterile media bottle.

3.3.2 Using a 1ml syringe and a 21 gauge x 1.5″ needle, aspirate 1ml of DPBS from the sterile bottle. Puncture the rubber lid and expel the contents of the syringe into the vial containing the powdered hCG hormone (stored in freezer or refrigerator according to brand).

3.3.3 Gently invert the vial 3-4 times to dissolve powder.

3.3.4 Using the same syringe, aspirate the hormone solution and expel it into the sterile bottle containing DPBS.

3.3.5 Aspirate 1ml of the diluted hormone from the sterile bottle and inject it into the empty hormone vial. Invert the vial, then aspirate the solution and expel it into the sterile bottle.

3.3.6 Repeat step 3.3.5 x 3 times per vial. This ensures that as much as possible of the powdered hormone is washed out of the ampoule.

3.3.7 Repeat steps 3.3.2 - 3.3.6 for the desired number of vials of the powdered hormone required.

3.3.8 Invert the sterile bottle several times to ensure the solution is completely mixed.

3.3.9 Using a P10 Gilson pipette and a 10ml Diamond pipette tip, aspirate 10ml of the hormone solution into a sterile universal tube and label 'hCG (date) for pH and osmolality testing'.

3.3.10 Aliquot the hormone solution into either;

- 1.4 ml into 1.5ml Eppendorfs (this will treat approx. 8x mice)

  and/or

- 4.6ml into 20ml Universal tubes (this will treat approx. 25x mice).

3.3.11 Apply labels on the Eppendorfs and Universals stating "hCG", "strength e.g 5iu" and "date".

3.4 Storage/testing

3.4.1 Store in the -20°C freezer until tested. **As soon as possible ensure that the new batch of hormones is tested before use.**

3.4.2 Conduct a pH (pH 7-7.2) and osmolality (270-290mOsm/kg) assessment on the hormone solutions in the universal tubes set aside for this purpose. Discard batch of hormones if the values are outside normal ranges.

3.4.3 Test each batch of hormone before use, on a cage of females scheduled for IVF. Ensure that PMS and hCG are not tested on the same females.

3.4.4 To ensure a timely supply of hormones, endeavour to prepare a new batch of hormones soon after the previous batch has started being used.

3.4.5 Shelf life once frozen is approx. 6 months.

3.4.6 Once aliquots have been thawed they must be discarded if not used.

3.4.7 Record the number of clutches and number of oocytes per female for test and control batches of hormones.