PREPARATION OF MEDIUM

Transport Saline (0.9%)

Prepare 0.9% saline (9 g NaCl per liter) in double distilled water and autoclave. Label as "Sterile 0.9% saline, date made" and store indefinitely at 4°C.

Oocyte Collection Medium (OCM)

1. Dissolve TCM-199 powder (without phenol red, and without glutamine) (Hyclone) for 10 L and 3.50 g $NaHCO_3$in 9 L ddH_2O. Add 100 ml Pen-Strep, Adjust pH to 7.2-7.4 and bring volume to 10 L. Sterile-filter 400 ml medium into 500 ml bottles and keep indefinitely at 4°C. Labels should read "Oocyte Collection Medium, - Supplements and date made".

2. On night before use, add the following: 1 aliquot of stock 4: BSS+Hep and 1 aliquot of stock 11: glutamine (4 ml). Change label to "+supplements", change date and use within a week.

Oocyte Maturation Medium

1. Prepare 44 ml aliquots of TCM-199 in 50 ml sterile tubes and store at 4°C until use.

2. On night before use, add the following to an aliquot of TCM-199:
   1 aliquot stock 3: BSS
   500 µl stock 8: gentamicin
   63 µl stock 6: Folltropin
   100 µl stock 5: estradiol
   500 µl stock 2: Na Pyruvate
   500 µl stock 25: Glutamax

Change label to read "Oocyte maturation medium" and use within 1 week.

TL Solutions - For Making TALPs

Note that these media can also be purchased from Caisson, Millipore or Lonza (formerly Biowhittaker).

1. To prepare media, mix the ingredients as described in Table 1 (all volumes are in milliliters), adjust the pH, check osmolarity (if osmometer is available) and sterile-filter the solution.

2. Write expiration date on the label (use within one week) and store at 4°C.

Table 1. Recipes for preparation of TL solutions

Ingredient	Sp-TL	HEPES-TL	IVF-TL
Water (ml)	79.232	177.0	40.157
Stock 17: NaCl (ml)	4.34	10.0	2.5
Stock 18: KCl (ml)	1.96	4.0	1.0
Stock 19: bicarb (ml)	10.00	1.6	5.0
Stock 20: phosphate (ml)	1.0	2.0	0.50
Stock 1: Na-lactate (ml)	0.368	0.372	0.093
Stock 21: HEPES (ml)	1.0	2.0	0
Stock 22: Ca chloride (ml)	1.0	2.0	0.50
Stock 23: Mg chlor (ml)	1.10	1.0	0.25
pH	7.4	7.3	7.4
Osmolarity (mOsm)	295-305	275-285	290-300

TALP (Tyrode's Albumin Lactate Pyruvate) Media

Recipe 1: using media from Caisson. Click for the formulation.

1. To prepare media, mix the ingredients as described in Table 2 and sterile-filter the solution.

2. Write expiration date on the label (use within one week) and store at 4°C.

Table 2. Recipes for TALP media using Caisson media.

Ingredient	Sp-TALP	HEPES-TALP	IVF-TALP
TL (ml)	76.0***	500.0	100.0
BSA, Fract V (g)	0.48	1.5	0
BSA, EFAF (g)*	0	0	0.6
Stock 2: pyruvate (ml)	4.0	5.0	1.0
Stock 8: gentamicin (μl)	160	750	100
Stock 7: heparin (μl)	0	0	500***

Recipe 2: using Millipore (Specialty Media) media

1. To prepare media, mix the ingredients as described in Table 3 and sterile-filter the solution.

2. Write expiration date on the label (use within one week) and store at 4°C.

Table 3. Recipes for TALP Media using Specialty Media media.

Ingredient	Sp-TALP	HEPES-TALP	IVF-TALP
TL (ml)	38.0***	100.0	50.0
BSA, Fract V (mg)	240	300	0
BSA, EFAF (mg)*	0	0	300
Stock 2: pyruvate (ml)	2.0	1.0	0.5
Stock 8: gentamicin (μl)	80	150	50
Stock 7: heparin (μl)	0	0	250***

* BSA, EFAF=essentially fatty-acid free Fraction V
** Save the 12 ml remaining after preparation of medium since it can be used for Percoll preparation.

*** The optimal heparin concentration will vary between bulls - if one bull is used frequently, test various concentrations of heparin in a preliminary experiment.

10X SP-TL *this is not really the same recipe for Sp-TALP above but is what we use for making up Percoll.*

1. Prepare 10x SP-TL stock solution by dissolving the following in 100 ml water:
   NaCl - 4.675 g
   KCl - 0.23 g
   $NaH_2PO_4+H_2O$ - 0.40 g
   HEPES 2.38 g

2. Adjust pH to ~7.3, sterile filter and store indefinitely at 4°C.

90 % Percoll

1. Place 4 ml of 10X SP-TL in a small beaker and add 0.084 g sodium bicarbonate and 90 ml Na lactate (Stock 1).
2. Stir until bicarbonate dissolves.
3. Add 36 ml Percoll.
4. Add 158 μl $MgCl_2$ (Stock 12) and 78 μl $CaCl_2$ (Stock 13).
5. While stirring, adjust pH to 7.3-7.45 and filter with a 0.45 μm filter (50 ml filter tube or similar bottle-top filter). If a precipitate forms in the Percoll solution, continue to stir. If compounds do not re-dissolve, then start over.

It is very easy to get precipitation if acid or base is added too rapidly during the adjustment of pH. Therefore, it is recommended that this step be done slowly.

KSOM-BE (Potassium Simplex Optimized Medium – Bovine Embryo Modification 2)

Recipe 1 – using KSOM+AA from Caisson (without pen/strep). Click for the formulation.

1. Purchase KSOM from Caisson Laboratories and store frozen. Once thawed, keep at 4°C for 2 weeks.

2. Add 1 ml pen/strep to a new bottle of thawed KSOM.

3. To 5 ml of KSOM stock add:

   EFAF BSA - 15 mg (3.00 mg/ml)

   Gentamicin stock 8A - 2.5 μl (0.5 ml/ml)

   Nonessential amino acids, 100X - 25 μl

4. Sterile filter medium through a 0.22 mm syringe filter into a sterile 10 ml beaker. Use immediately.

Recipe 2 – using KSOM from Millipore (contains pen/strep)

1. Purchase KSOM MR-106-D from and store frozen. Use with caution after the expiration date provided by the manufacturer. Once thawed, keep at 4°C for 2 weeks.

2. To 5 ml of KSOM stock add:
   EFAF BSA - 15 mg (3.00 mg/ml)
   Gentamicin stock 8A - 2.5 μl (0.5 μl/ml)

Nonessential amino acids, 100X - 25 μl

Note: This formulation is different than was previously described on this Webpage. Cell and Molecular Technologies has developed a new KSOM formulation that contains essential amino acids and half the required concentration of nonessential amino acids.

3. Sterile filter medium through a 0.22 mm syringe filter into a sterile 10 ml beaker. Use immediately.

CR1aa (an alternative culture medium)
Note: the patent for this medium is held by Infigen

1. Make CR1 stock (prepare in a 100 ml volumetric flask):

NaCl	0.670 g
KCl	0.023 g
NaHCO3	0.220 g
Na Pyruvate	0.004 g
Glutamine	0.015 g
Hemi-Ca Lactate	0.055 g

Add first 5 ingredients to volumetric flask. Add water (~90 ml). Thoroughly dissolve constituents and then add Hemi-Ca Lactate. Add remaining water. Store for up to 2 days at 4°C.
Note: constituents of this medium are known to precipitate out of solution. To minimize the chances of this occurring, make sure all constitutents are dissolved before adding hemi-Ca lactate and use immediately after making. If a medium appears white and cloudy, discard and start again.

2. To prepare CR1aa, add the following to 5 ml of CR1 stock:

EFAF BSA - 15 mg (3.00 mg/ml)
Gentamicin stock 8A - 2.5 μl (0.5 μl/ml)
Nonessential amino acids, 100X - 50 μl
Essential amino acids, 50X - 100 μl

Sterile filter medium through a 0.22 μm syringe filter into a sterile 10 ml beaker. Use immediately.

modified Synthetic Oviduct Fluid (m**SOF)**

This is based on the formulation of Ficher-Brown et al. Zygote 10:341-348 (2002) except that the concentration of Na-lactate and BSA are altered, the BSA is essentially fatty acid free BSA, and ALA-glutamine, Na-citrate, and myo-inositol were added. We purchase the medium as a custom formulation from Millipore Specialty Media (BSS0771) and supplement the medium.

To prepare medium, mix the ingredients and sterile-filter the solution. Write expiration date on label (use within 3 weeks) and store at 4° C.

Recipe:

Specialty Media SOF	9.3	mL
Gentamicin Sulfate (stock 8)	50	µL
200x Na Pyruvate (0.0440 g/5 mL Sigma H_2O; expires 1 month)	50	µL
100x Myo-Inositol (0.50 g/10 mL Sigma H_2O; expires 3 months)	100	µL
100x Na Citrate (0.1 g/10 mL Sigma H_2O; expires 3 months)	100	µL
100x ALA-Glutamine (0.217 g/10 mL Sigma H_2O; expires 3 months)	100	µL
Na Lactate Syrup	2.83	µL
MEM Non-Essential Amino Acids	100	µL
BME Essential Amino Acids	200	µL
*Essentially Fatty Acid Free BSA	0.04	g

*PVA (0.01 g) can be substituted for BSA

Specialty Media SOF			**m-SOF**		
NaCL	107.70	mM	NaCL	107.7	mM
KCl	7.16	mM	KCl	7.16	mM
KH_2PO_4	1.19	mM	KH_2PO_4	1.19	mM
$CaCl_2$ $2H_2O$	1.17	mM	$CaCL_2$ $2H_2O$	1.17	mM
Na-Lactate	3.30	mM	Na-Lactate	5.30	mM
$NaHCO_3$	25.07	mM	$NaHCO_3$	25.07	mM
$MgCl_2$ $6H_2O$	0.49	mM	$MgCl_2$ $6H_2O$	0.49	mM
			ALA-glutamine	1.00	mM
			Na-Pyruvate	0.40	mM
			Tri Na-Citrate	0.50	mM
			Myo-Inostitol	2.77	mM
			Gentamicin Sulfate	25.00	µg/mL
			MEM Non-Essential Amino Acids	10.00	µL/mL
			BME Essential Amino Acids	20.00	µL/mL
			*Essentially Fatty Acid Free BSA	4.00	mg/mL
			*PVA (1 mg/mL) can be substituted for BSA		

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