Syllabus for Licensing Examination of M.Sc. Medical / Clinical Embryology

Nepal Health Professional Council

Bansbari, Kathmandu

Table of Content

1. Theory

A. Basic skill in laboratory (BSL)

- Good laboratory practice, personal attitude, communication and confidentiality
- Laboratory mouse: handling, experimentation and maintenance of colony
- Monitoring estrous cycle, superovulation and mating
- Animal handling, injection and dissection
- Reading research papers, documentation of research data and presentation
- Journals in Reproduction, online resources in Embryology
- Statistical evaluation of data
- Washing and preparation of glassware for culture work
- Microscopes: principles, application and care
- Waste disposal
- Sterilization and disinfection
- Laboratory chemicals: handling, preparation, storage, stability and hazard
- Handling of equipment and maintenance
- Pipetting & troubleshooting

B. Basic Reproductive Biology Module

- Structure of eukaryotic cell
- Meiotic and mitotic cell division
- Somatic and germ cell
- Regulation of cell cycle
- Research methodology I
- Research methodology II
- Ethics in research
- Anatomy of male reproductive system
- Anatomy of female reproductive system
- Histology and function of male reproductive system
- Histology and function of female reproductive system
- Hormones: mechanism of action and regulation
- Reproductive hormones
- Endometrial & Implantation Endocrinology
- Advanced Hormonal concepts & Biomarkers
- Genomics, proteomics & metabolomics
- Structure of spermatozoa, oocyte and embryo
- Spermatogenesis
- Epididymal sperm transport and maturation
- Regulation of spermatogenesis
- Menstrual cycle
- Oogenesis
- Folliculogenesis
- Sperm transport in male

- Sperm, oocyte and embryo transport in female
- Follicle and embryo transport in female
- Capacitation
- Acrosome reaction
- Sperm-zona interaction
- Fertilization
- Errors in fertilization
- Physiology of implantation
- Hormonal regulation of implantation
- Fundamentals of immunology
- Immunological basis of infertility
- Regulation of gene expression
- Introduction to human genetics
- Genetic abnormalities in male reproduction
- Genetic abnormalities in female reproduction
- Epigenetics in reproduction
- Stem cell Biology and Regenerative Medicine
- Reproductive toxicology – male
- Reproductive toxicology – female
- Role of apoptosis in development
- Early morphogenesis and patterning
- Impact of microbial contaminants on gametes and embryos development in Andrology and IVF laboratory.
- Bioinformatics in IVF

C. Andrology Module

- Setting up of an Andrology laboratory
- Safety in Andrology laboratory
- Quality control in andrology
- Semen analysis
- Computer assisted semen / sperm analysis (CASA)
- Biochemistry of spermatozoa and seminal plasma
- Predictive value of semen analysis
- Antisperm antibodies
- Sperm wash techniques
- Seminal microbiology and virology
- Semen cryopreservation, donation and banking
- Therapeutic insemination procedures
- Sperm fertilizing ability testing
- Sperm DNA integrity testing: clinical utilities
- Male contraception
- Testicular disorders and infertility
- Disorders of erection and ejaculation

- Clinical approach to Patients /couples consultation and counseling on semen quality, Sperm disorders and potential treatment approaches.
- Semen /sperm abnormalities and their treatment options like: Different types of Sperm washing, cell sorting (Microfluidics), using theophylline/Pentoxyphyline, IUI, Conventional IVF, ICSI, PICSI, IMSI, etc.
- Ethics and ethical issues in Andrology

D. Embryology Module

- Oocyte abnormalities, selection and treatment
- Early embryo and blastocyst development
- Role of maternal and paternal factors on embryo development
- Embryonic genome activation
- Embryo metabolism – I
- Embryo metabolism – II
- Embryo grading
- Assessment of embryo viability
- Development of gonads
- Gastrulation and neurulation
- Placenta
- Cell to cell communication in embryo development
- Teratogenesis
- Twinning
- Therapeutic cloning
- Embryonic stem cell
- Metabolomic screening of embryos to enhance successful selection
- Ethical issues in embryology
- Multi-nucleation in embryos
- Clinical counseling on ovarian follicular development and oocyte-related abnormalities.
- Follicles / oocytes and embryo abnormalities and their treatment options like: LAH, calcium ionophore, Spindle microscopy etc.

E. Assisted Reproductive Technology (ART) Module

- Setting up of an ART laboratory
- Laboratory management, quality control and ethics
- Supply Chain, Disaster Management & Preventive Maintenance
- ART services, key performance indicators (KPIs) & ART research methodology in IVF
- Bioethics & Legal Compliances in IVF/ART
- Clinical consultation on sperm, oocyte abnormalities, ART treatment strategies, and embryo quality and embryo transfer.

- Intrauterine Insemination (IUI)
- In vitro fertilization (IVF)
- Intracytoplasmic sperm injection (ICSI) & PICSI
- Intra morphologically selected sperm injection (IMSI)
- Embryo culture system
- Culture medium
- Embryo grading
- Laser assisted hatching (LAH)
- Embryo cryopreservation
- Oocyte cryopreservation
- Ovarian and testicular tissue cryopreservation
- Ethical issues in semen banking
- Metabolomics and its applications in ART
- In vitro maturation (IVM)
- Health economics in ART
- Air quality in IVF lab
- Oxidative stress in assisted reproduction
- Embryo transfer
- Principles of aseptic procedures
- In vitro systems and their improvement
- Quality control procedures
- Embryo biopsy techniques
- Preimplantation genetic screening/diagnosis (PGS / PGD)
- Preparation of guidelines for IVF / ART
- National & International guidelines for IVF / ART
- Laboratory protocols & troubleshooting
- Risk management in IVF lab
- Data management and interpretation

F. Clinical Modules

- Infertility counseling
- Male factor infertility
- Ultrasound (including Doppler) and its application in ART
- Superovulation regimes
- Ovarian hyperstimulation and its management
- Oocyte pickup, embryo transfer and embryo reduction
- Luteal phase support

2. Diagnostic and Treatment options (Clinical Protocols)

A. Andrology and Semenology: "WHO Laboratory Manual guidelines for examination & processing of human semen", 6th Edition

- **Routine Semen Analysis (Macroscopic & Microscopic):**
  - Macroscopic: assessment of sample liquefaction time, appearance, volume measurement, viscosity (consistency) and p^H testing.
  - Microscopic: Sperm concentration & count, assessment of different types of motility, motility index, vitality, Hypo-osmotic swelling (HOS) test, sperm morphology, functional sperms, velocity and to evaluate strict criteria for normal vs abnormal head, necks, mid-piece, and tails, trial wash

- **Advanced Diagnostic and Functional Andrology**
  - Sperm DNA Fragmentation (SDF) Assays: Sperm Chromatin Dispersion (SCD) test or TUNEL assay, and other advance assays related to sperm qualities.
  - Differential morphology, trial wash, DFI etc., to decide the potential treatment options
  - Leukocytospermia Quantification: Staining and quantification of round cells vs white blood cells using peroxidase (Endtz) test.
  - Anti-sperm antibody (ASA) screening: Performing screening assays such as the Mixed Antiglobulin Reaction (MAR) test or immunobead test.
  - Retrograde Ejaculation Analysis:Processing and isolating sperm from post-ejaculatory urine samples.
  - Protamine Replacement:
    Cromomycin A3 (CMA3) Staining: A fluorescent dye that competes with the protamines to bind to DNA. Higher fluorescence (CMA3)-positive) shows low protamine levels and poor chromatin packaging.
    - ✓ Aniline Blue Staining: A dye that stains basic proteins to detect excessive residual histones. It highlights incomplete chromatin maturation and failed replacement.
    - ✓ Protamine 1 and 2 (P1:P2): Measure ratio of protamine 1 and protamine 2 by high-performance liquid chromatography (HPLC) which is often abnormal in infertile men.
    - ✓ Mass Spectrometry: Advance Proteomic analysis used in research setting to detect specific protamine isoforms and post-translational mmodification like Phosphorylation

- Sperm Preparation & Processing Techniques
  - Density Gradient Centrifugation (DGC): Preparing discontinuous layers (e.g., 40 /45 % and 80/90 %)to isolate high-quality, motile sperm from contaminated or poor –quality ejaculate.
  - Swim –up techniques: Standard direct swim-up from semen or from a washed pellet for normozoospermic samples.
  - Processing Difficult Samples: Specialized protocols for processing severe oligozoospermia, teratozoospermia, or highly viscous samples.

    - Surgical Sperm Retrieval Processing: Extraction isolation of viable sperm from testicular tissue (TES/TESE) or epididymal fluid (PESA/MESA).
- Cryopreservation & Bio-banking
    - Sperm freezing Protocols: Proper mixing ratios with glycerol-based cryoprotectants, vapor phase freezing, and plunging into liquid nitrogen.
    - Specialized Freezing: Techniques for cryopreserving single or low numbers of sperm (e. g., microdroplets or specialized carriers).
- Laboratory Management & Quality Control (QC)
    - Equipment Calibration: Daily calibration and temperature tracking of heating blocks, microscopes and centrifuges.
    - Internal Quality Control: Utilizing preserved beads or running duplicate controls for manual counting validation.
    - Safety and Hazard management: Handling potentially infectious samples, enforcing universal bio-safety precautions, and managing liquid nitrogen safety.

B. Embryology:

- **Laboratory Setup, Ambient Control & QA**
    - Incubator Calibration: Hands-on monitoring and validation of temperature, Co_2, O_2 level using external gas analyzers and digital thermometers.
    - Workstation and IVF room Validation: Verifying laminar flow velocity, HEPA filter efficiency, and VOC levels using specific air-quality meters, air particles in IVF room, etc.
    - Culture Dish Preparation: Preparing micromanipulation, conventional IVF, and culture drops under sterile mineral oil overlay with proper equilibration times.
- **Follicles and Oocytes Processing and Handling**
    - Follicles Retrieval (OPU Processing):Scanning follicular aspirates under a stereozoom microscope to isolate cumulus-oocyte complexes (OCCs)
    - Follicles Denudation: Mechanical stripping of cumulus and corona radiate cells using enzymatic (hyaluronidase) and mechanical (Pipetting) methods
    - Maturity Assessment: Microscopic evaluation to grade oocytes as Germinal vesicle (GV) Metaphase I (MI), or mature metaphase II (MII).
- **Fertilization & Micromanipulation (ICSI)**
    - Conventional Insemination: calculating and executing exact insemination concentrations for standard IVF drop setups and maturity of oocyte.
    - Micromanipulator Alignment: Setting up, centering, and calibration holding and injection pipettes on an inverted microscope.
    - ICSI Execution: Practicing sperm selection (PVP) immobilization) and mechanical microinjection techniques on training models.
    - Fertilization Check: Microscopic inspection at 16-18 hours post-insemination to identify normal fertilization (2 PN, two polar bodies) vs abnormal cases (1 PN, 3 PN).
- **Embryo Culture & Morphokinetic Grading: Cleavage Timeline**
    - Zygote to Cleavage-Stage Evaluation (DAY 0 to 2 & 3):
    - 24 to 30 hours: single celled zygote divides mitotically for the first time into 2-cell embryo making the start of cleavage.

- 40-50 hours (Day 2): Cell divides again to form a 4-cell embryo.
- Approx. 72 hours (Day 3): Continued division results in 8-16 cells called Day 3 cleavage cell.
- In approx. 96 hours (Day 4): Structural transition into Blastocyst before uterine implantation.
- Grading blastomere count, cell symmetry, and fragmentation percentages.
- Blastocyst Evaluation (Day 5 & 6): Applying different grading systems to evaluate blastocoel expansion, the inner cell mass (ICM), and trophectroderm (TE).
- Time – Lapse Data Interpretation: Reviewing annotations and morphokinetic milestones using continuous monitoring software.

- **Cryopreservation & Vitrification**
  - Embryo Vitrification: Performing precise equilibration steps in increasing concentrations of cryoprotectants (EG/DMSO) and executing ultra-rapid cooling on open or closed carrier systems.
  - Warming Protocols: Performing step-down dilution warming steps to remove cryoprotectants safely.
  - Survival Assessment: Evaluating post-thaw blastomere survival and blastocyst re-expansion metrics to predicts embryo viability, implantation potential, and live birth rates.

C. Assisted Reproductive Technology (ART) Services and ART Research Methodologies in IVF:

- **Standard Operating Procedures (SOPs) Lab Logistics**
  - Patient Workflow Mapping: Simulating end-to-end laboratory sequence from oocyte pickup (OPU) scheduling to embryo transfer or cryopreservation.
  - Double Witnessing systems: Completely eliminate sample mixing hazards
    - ✓ Manual dual-signing system
    - ✓ Electronic radio frequency identification (RFID) or
    - ✓ Barcode tracking
  - Incident Reporting and Disaster Management: simulating laboratory emergencies, including gas line drops, incubator element failures, power outages, and liquid nitrogen tank leaks.
- **Clinical Documentation, Electronic Health Records (HER) & Auditing**
  - Embryology Logbook: hands-on data entry for patient history, stimulation cycles, follicle counts, fertilization outcomes, and developmental tracking sheets.
  - **Key performance Indicator (KPI):** Weekly calculation and clinical graphing of essential lab metrics:
    - ✓ Oocyte Denudation Damage Rate: target < 5%
    - ✓ Normal fertilization Rate (2PN): target >75% for ICSI
    - ✓ Blastocyst Development Rate: Target > 50%
    - ✓ Post – Thaw Embryo Survival Rate: Target >75%

ART Research Methodologies in IVF

Experimental Design & Laboratory Models

- Animal / Alternative Training models: Utilizing mouse embryos or non-human models (where ethically approved) for micromanipulation training, optimization testing, and media efficacy trials.
- Media Condition Comparisons: Designing split-oocyte cohort experiment to test variations in sequential vs single-step culture media behaviors.

Biosensor Validation & Non-Invasive Biomarker Assays

- Spent Media Analysis: Collecting and processing micro-droplets of spent embryo culture media for metabolic analysis (e.g., glucose consumption profiling, amino acid turnover) or non-invasive prenatal testing for aneuploidy (niPGT – A).
- Advance equipment Testing: Using particle counters, Volatile organic compound (VOC) meters, and ultra-fine p^H meters to measure sub-lethal environmental fluctuations across different incubation settings.

Data Management & Biostatistics in ART

- Statistical Modeling for Clinical Outcomes: Setting up software arrays (e.g., SPSS, R, or Excel plugins) to compute correlation coefficients between clinical variables (such as maternal age, sperm DNA fragmentation index) and final clinical pregnancy rates.
- Journal Club & Research Auditing: Practical cross-examination of contemporary embryology literature, checking control cohort validations, sample size powers, and confounding parameters.

D. Cytogenetics:

- **Preimplantation genetic testing(PGT) Biopsy Techniques:**
  - Cleavage-stage biopsy (Day 3 Embryos): mechanical or laser-assisted breaching of the zona pellucida to safely aspirate a single blastomere.
  - Blastocyst Trophectoderm Biopsy (Day 5/6 Embryos): Advanced micro-surgical sampling of 5-10 trophectoderm cells without disrupting the inner cell mass (ICM).
  - Biopsied conditioning: Rinsing, loading, and cellular lysis of the sampled cells into micro-tubes (TUBING) for downstream genetic analysis
- **Advanced Molecular Cytogenetic (PGT Analysis):**
  - **Next Generation Sequencing Hybridization (FISH):** Interpretation of NGS sequencing library data to screen for embryo aneuploidies(**PGT – A**), structural rearrangements (**PGT – SR**), or single gene disorders (**PGT – M**)
  - **Fluorescence in situ Hybridization (FISH):** Preparation of blastomere slide or lymphocyte, probe hybridization, and signal enumeration under a fluorescence microscope to detect mosaicism or numeric anomalies.
  - **Polymerase chain reaction (PCR) & Karyotyping:** Applied techniques for checking genetic markers, detecting contamination, and tracking targeted inherited conditions.

- **Classical Cytogenetic & Karyotyping:**
  - Slide Preparation & Chromosome spreading
  - Peripheral blood lymphocyte culture
  - G-Banding & Karyotype Analysis
- **Clinical Case interpretation & Reporting**
  - Embryo Mosaicism Decision Matrix: reviewing and interpreting PGT reports to categorize embryos as euploidy, aneuploidy, or deciding transfer priority.
  - ISCN Nomenclature Practice: Writing and deciphering cytogenetic results using the Standard International System for Human Cytogenetic Nomenclature (ISCN).

E. Laboratory management, quality control & ethics

- **Cleanroom Engineering & Air Quality Infrastructure (HVAC / HEPA)**
  - Zoning and pressure Cascades: Practical testing of different air pressure gradients between the patient procedure room (OPU), embryology laboratory (cleanest zone), and washing areas.
  - Volatile Organic Compound (VOC) Management: Measuring and managing ambient molecular contamination using photoionization detectors (PID sniffer meters), practicing VOC – free painting, cleaning, and material entry protocols.
  - Particle Count Validation: Hands-on operation of airborne particle counter to verify ISO Class 7 (Class 10000) and ISO Class 5 (Class 100) work areas. ISO class 5 is 100 times cleaner than ISO class 7 based on maximum allowance of airborne particles per cubic meter.
    Particle concentration limits (at ≥ 0.5µm)
    ISO Class 5: Allows a maximum of 3,520 particles per cubic meter (m^3)
    ISO Class 7: Allows a maximum of 352,000 particles per cubic meter (m^3).

- **Supply Chain, Disaster Management & Preventive Maintenance**
  - Liquid Nitrogen (LN_2) Tank Infrastructure: Managing dewars, monitoring cryogenic liquid levels, calculating consumption rates, and executing safe tank –to-tank biological material transfers.
  - Emergency Power & Gas Backup Drills: Routine testing of uninterruptible power supply (UPS) systems, automated backup generators, and gas line manifold swap-overs (CO_2, O_2, and N_2).
  - Consumable Batch Testing (BIO-assays): Verifying certifications for Embryo Toxicity testing (MEA – Mouse Embryo Assay) and Endotoxin Testing (LAL – Limulus Amoebocyte Lysate) before releasing clinical lots.

Quality Control (QC) & Quality Assurance (QA)

- **Daily Equipment Calibration & Environmental Monitoring**
  - Temperature Mapping: Using high-precision digital probes (calibrated to ± 0.1°c) to map variations across work surfaces, heating blocks and incubator shelves.
  - Gas & p^H Metrics: Measuring incubator gas metrics using external infrared CO_2 / O_2 analyzers. Calibrating benchtop and continuous – monitoring p^H meters using standard reference buffers.

- Microscope Alignment: Performing Kohler illumination adjustment and micromanipulator laser alignment checks.

- **Key performance Indicators (KPIs) and Data Auditing**
  - Vienna Consensus Alignment: Setting up laboratory data arrays to track mandatory global benchmarks.
  - Statistical Performance Graphing: Utilizing Levey-jennings chart and moving average rules to detect lab drift or sudden drops in critical milestones (e. g., fertilization rates, blastocyst conversion rates, or clinical pregnancy rates)

Bioethics & Legal Compliance in IVF

- **Double-witnessing & Electronic Traceability**
  - Manual Chain –of –Custody: Executing a **strict two- clinical embryologist** cross-verification protocol for identify validation at every critical gamete transition stage:
    - ✓ Oocyte Retrieval (OPU)
    - ✓ Semen collection and processing
    - ✓ Insemination / Microinjection (ICSI / IMSI)
    - ✓ Embryo transfer (ET)
    - ✓ Cryopreservation (Vitrification & Warming/Thawing)
  - Electronic Tracking Setup: Simulating automated electronic witnessing using barcodes or Radio Frequency Identificaion (RFID) chip tags on culture dishes.
- **Consent, Documentation & legal Boundaries**
  - Audit of Infertility Eligibility: Documenting valid, verified diagnostic files to confirm a clinical infertility diagnosis before starting treatment, according to national medical protocols
  - Identity Dossier Management: verifying legal marriage credentials or passports for international patients to prevent statutory compliance violations.
- **National Regulatory Boundaries (Nepal Context)**
  - Gamete Donation Tracing: Creating secure databases to monitor strict legal thresholds for gamete donor:
    - ✓ Oocyte Donors: Age 20-35, maximum 6 times cycles, minimum 3-months gaps.
    - ✓ Sperm Donors: Age 20-35, Maximum 10 successful live-birth cycles, minimum 15-days collection recovery windows.
    - ✓ In Nepal, the application of Pre-implantation Genetic Testing (PGT) operates under strict ethical, legal, and medical boundaries. Medical guideline and legal frameworks restrict its use to prevent severe genetic disorders, explicitly banning non-medical sex selection.

Recommended Text Books, journals & Research Papers:

1. Eberhard Nieschlag, Hermann M. behre, Susan Nieschlag (2010). Andrology: Male Reproductive Health and Dysfunction (3rd ed.). (springer).
2. Bruce Alberts, Alexander Johns, Julian Lewis (2008). Molecular Biology Of THE CELL 5th ed.).
3. Kim E. Barrett, Susan M. Barman, Scott Boitano (2010). Ganong's review of Medical Physiology (23rd ed.).
4. David k. Gardner, Botors R. M. B. Rizk (2011). Human Assisted Reproductive Technology
5. David K. Gardner, Michelle Lane, Andrew J. Watson. A Laboratory Guide to the Mammalian Embryo.
6. Rechard E. Jones, Kristin M. Lopez (2006). Human Reproductive Biology, 3rd Edition
7. Arthur C. Guyton & John E. Hall (2006). TEXT BOOK OF Medical Physiology (11th ed.).
8. Jimmy D. Neill and Knobil (2015). Physiology of Reproduction (4th ed.).Volume 1 & 2
9. Kay Elder and Brian Dale (2011). In – Vitro Fertilization (3rd ed.).
10 Langman's Medical Embryology (8th ed.).
11. THE ASSISSTED REPRODUCTIVE TECHNOLOGIES (REGULATION). RULES-2022, ICMR
12. David K. Gardner (2006). In Vitro Fertilization: A Practical Approach
13. Steven D. Fleming and Robert S. King (2003). Micromanipulation in Assisted Conception: A user's Manual and Troubleshooting Guide.
14. Zsolt Peter Nagy, Alex C. Varghes, Ashok Agrawal. Practical manual of In vitro Fertilization.
15. Christopher De. Jonge and Christopher Barratt. The Sperm Cell: Production, Maturation, Fertilization, Regeneration.
16. WHO laboratory manual for the Examination and processing of human semen (6th ed.).
17. WHO Laboratory Biosafety manual, 2004
18. Thomas Hunt Morgan. Embryology and Genetics
19. G. P. Pal. Medical Genetics
20. Richard E. Jonesand Kristin H. LopezHuman Reproductive Biology (4th ed.).
21. M. Cristina Malgi, Gayle M. Jones et al.,(2012) Atlas of Human Embryology: From Oocyte to Preimplantation Embryos. Journal *of Human Reproduction*.
22. Yujing Li (Editor- in -chief) Journal of *Human Genetics & Embryology*
23. Several Journals and online Research Papers.