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REVIEW ARTICLE

Chicken Primordial Germ Cells (PGCs) Formation, Regulation, and Fate Determination under OMICS Approaches: A Comprehensive Scientific Review

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A B S T R A C T

Chicken is a rich source of protein in food and model animal for research due to ease of handling, low generation interval, and early sexual maturity. Genetic manipulation to study genetic role could improve chicken genetics, and reproductive biology. As precursor of germ cell formation and new generation development, primordial germinal cells (PGCs) can be studied using molecular biology methods like gene editing to precisely modify the next generation. OMICS has four categories; Metabolomics is involved in providing energy to PGCs by nutrition and controlling PGCs by different metabolomics pathways. Genomics, expression of genes, genetic markers, and epigenetic regulators are involved in the process of control of PGCs. Transcriptomes is the influence of genomics in the form of message or code controlling PGCs by different pathways. Proteomics is the last and major part involving the formation of different proteins responsible for the regulation of PGCs. Integrated OMICS improves PGCs regulation, genetics selection, and reproduction management, but the large amount of data from all fields limits it. In developmental biology, germline modification, germ cell transplant, and PGCs with unique biological traits may produce the best transgenic chickens. Reproductive biology is slowly developing and can use gene-editing tools like CRISPR/Cas9 proteins and Single Cell RNA sequences for innovation.

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INTRODUCTION

Primordial Germ Cells (PGCs) are the embryonic ancestor of germ cells originating from the gonads. As in other avian species, PGCs are vital for reproduction and the genetic diversity in chickens. Avian PGCs arise at the earliest stage of ontogenesis, or during embryogenesis, and are primarily in the third stage of the avian embryo (Fig. 1A). Initially derived from the posterior epiblast. Stimulated by the environment and sex-determining mechanisms as well as by molecular factors like genomics, transcriptomics, proteomics, and metabolomics, to divide or develop them either in sperm or oocytes (Fig. 1D) (Niu *et al.*, 2024). PGCs also control cell fate determination by using the inherited information in the genes and signals that they receive from the micro-environment of the tissue (Ichikawa and Horiuchi, 2023). PGCs formation is a regulated spatial, sequential process that includes different signaling pathways, transcription factors, epigenetic factors, and their combined effects (Fig. 1C). Sex differentiation and gonadal development depends on PGCs (Kim and Han, 2018a). Working on PGCs in-vitro to produce reservoirs for avian breeds, genetic resources conservation, improving fertility and sexing mechanisms is enhanced. For example, we can induce and control histone methylase MII2 (Mixed-Lineage Leukemia 2) and Cvh (Chicken vasa homolog) regulators for histone methylation leading to improvement in PGCs production, *BLIMP1* indirectly controlling PGCs formation, and use as a gene bank (Zhang *et al.*, 2021). For germline genetic changes that create transgenic birds, PGCs are promising genetic engineering tools (Squires and Drake, 2022). Manipulation technologies in PGCs are appropriate tools for producing animals with specific desirable genes of disease resistance, reproduction, production , and other desirable traits (Han and Lee, 2017).

Over last decade, “OMICS” technologies improved in developmental biology focused on PGCs behavior in birds.

Metabolomics, genomics, transcriptomics, and proteomics each affect cell development and differentiation molecularly. Genomics studies complex genes and genomes. The chicken DNA study investigated gene origins for characterization, diseases, and metabolism. Genomic data helps us understand PGCs formation, migration, and differentiation. By studying genomics, researchers can identify PGCs-developing genes and regulatory factors (Luo *et al.*, 2022). Transcriptomics studies all genome-transcribed RNAs, including non-coding RNAc, microRNAs, mRNAs, rRNAs, tRNAs, and others. Temporal transcriptional program is a more classified parameter that relates to the gene activity program during PGCs formation from RNA levels. Some genes must be upregulated while others are downregulated at certain times, such as during PGCs specification or migration. In PGCs research, molecular profiles can help identify vital genes that regulate cell information and fate specification (Zuo *et al.*, 2022). Proteomics examines protein structures, interactions, functions, and modifications. Proteins perform biological processes in cells, so proteomics can reveal the PGCs molecular mechanism. Analyzing PGCs protein profiles helps identify signaling pathway, differentiation, and migration proteins. (Meng *et al.*, 2022). Metabolomics studies cell or organism metabolites. It can identify or quantify metabolites like amino acids, lipids, and sugars, and the cell metabolic state contains all molecular or cell-level metabolites for PGCs study. Metabolomics in PGCs research focusses on metabolic profiling changes during development and differentiation (Fig. 1 B)(Huang *et al.*, 2022). Integrated OMICS, Metabolomics integrated with genomics, transcriptomics and proteomics are useful in understanding the biological processes and their detailed regulation such as PGCs formation and fate determination. Genomics predict the presence of genes, transcriptomics reveals spatial expression of genes, proteomics provide information how gene performs their function, metabolomics can show metabolic patterns of a cell. Through the integration of this data assembled from these methodologies, researchers can define a model of PGCs specifying how they migrate and differentiate or reproduce sexually (Zuo *et al.*, 2022). This review uses "OMICS" data to understand avian PGCs development's molecular regulation. This review covers metabolic, genetic, and epigenetic control of PGCs specification, migration, differentiation, gene-level 'OMICS' origin, regulatory circuits, and signaling pathways. This review coordinates information from different fields for novel studies on PGCs' role in avian growth and reproduction, OMICS' role in PGCs development, migration, sex determination, future research "OMICS" strategies for other model systems, and germline epigenetic regulation.

Development and differentiation of PGCs in chickens embryo: Chickens at the embryonic stage are a unique model for studying the dynamics of early embryogenesis and PGCs formation. In chickens, PGCs are first observed by Waldeyer germen scientist, very early in development. In chickens, PGCs arise from one of the multipotent derivatives of epiblast or germ wall that is why called moniker "germ cells" marked by *Stella* (Developmental Pluripotency Associated 3) and *Vasa or* DDX4 **(**DEAD-box helicase 4) (Kim and Han, 2018b). PGCs should be well positioned in the genital ridges for further migration into the developing gonads (Sasanami, 2017). 'OMICS' technologies could be used for germline stem cell therapy and cell-based reproductive technologies improvement by providing knowledge of transcriptomics to highlight genes related to reproductive traits.

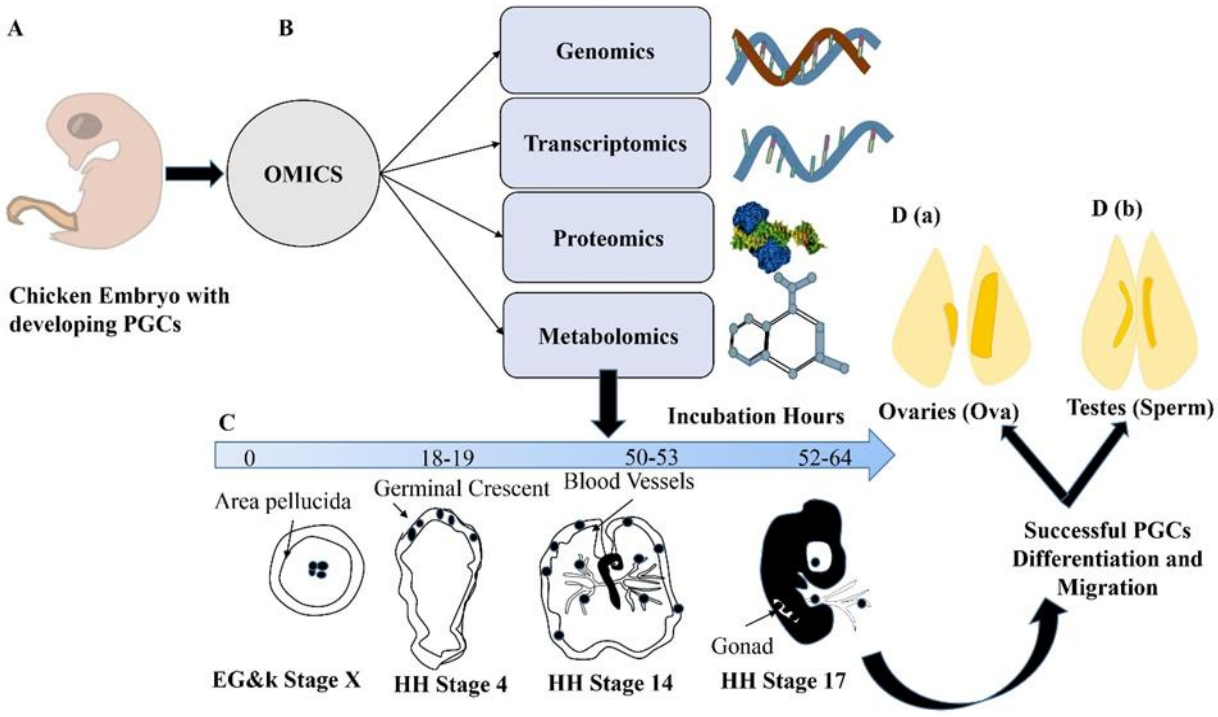

Fig. 1: OMICS Play key role in Formation of gonads, differentiation of PGCs into sperm or ova. Note: (A) Chicken Embryo at very earliest stage. (B) OMICS from genomics to transcriptomics, proteomics and metabolomics to control PGCs formation, Migration and Differentiation. (C) Illustration of stages for PGCs formation and during development and OMICS influence at these stages until PGCs migrate to gonads (Ichikawa and Horiuchi, 2023). (D) Goads receive differentiated form of PGCs (D (a)) PGCs colonize the gonads and develop into testis. (D (b)) PGCs colonize the gonads and develop into eggs.

Essential signaling pathways for PGCs formation, differentiation and migration: Multiple signaling pathways have been recorded to influence PGCs in different ways Wnt (Wingless/Integrated-1) signaling: which is recognized to play an important role in the formation of PGCs from epiblast. Glycogen synthase kinase-3 (GSK-3), a serine/threonine protein kinase, involved in Wnt/b-catenin, Sonic hedgehog & Notch signaling (Chen *et al*., 2020). Bone morphogenetic protein (BMP) signaling: involved actively in the early initiation of the PGCs. Fibroblast Growth Factor (FGF) signaling: also have an effect on signaling pathways as demonstrated that the FGF signaling pathway is required for both the migration and survival of PGCs (Fig. 2 A). Hamburger–Hamilton (HH) signaling: is necessary for adequate PGCs colonization of the gonadal region. HH signaling regulates the transcription of proteins PTCH2 (Patched 2) and C2EIP (Chromosome 2, Embryonic Inhibition Protein) for stem cell differentiation into PGCs (Zuo *et al.*, 2018). These are used to determine the pathways for PGCs specification, survival and maintenance while in migration.

Important genes for PGCs development: Genes playing a critical role in the generation of PGCs are as follows: in chickens many genes are critical for PGCs specification. *BLIMP1* **(**B lymphocyte maturation protein 1**)** provides a heterotypic barrier against tissues formed of epiblast cells, which continue to prevent its fate determination towards PGCs. *VASA:* A germline specific marker that aids in the germline germ cell, maintenance of germline fate and function. *STELLA:* This gene is required for the development and sustaining of PGCs through epigenetic silencing. These genes are linked to the epigenetic changes required to generate functional gametes from PGCs (Fig. 2 B) (Dehdilani *et al.*, 2023).

Molecular control of PGCs: PGCs formation may be controlled molecularly like chromatin modifications as a mode of epigenetic control of germ cells. DNA methylation, and histone modifications, methylation & acetylation (Fig. 2 C b & c) are the most well known epigenetic mechanisms. Chromatin remodeling controls the ability of cells to pass genes required for the formation of PGCs including *BLIMP1, VASA*, and *STELLA* while also epigenetically reactivating the somatic genome (Meng *et al.*, 2022). Epigenetic modification in the fate determination of chicken germ cells particularly activation of germline cells specific genes is associated with histone acetylation and DNA demethylation at PGCs promoters (Woo and Han, 2024a). There is still a knowledge gap in avian studies to determine the nature of epigenetic reprogramming. The epigenetic profile of chicken PGCs has been reported to contain histone changes, post-transcriptional control by short RNAs, and DNA methylation (Fig. 2 C a) (Woo and Han, 2024b). Through methylation, mammalian PGCs induce mono allelic expression of anchoring genes while also inactivating one of the two X chromosomes, suppressing gene expression, and maintaining the inactivated retrotransposons (Mathan *et al.*, 2023).

Metabolomics and its implications to PGCs specification: Metabolomics is the wide-ranging identification and measurement of the small biomolecules called metabolites. Conducting measurements of metabolites, from biological samples involves the use of mass spectrometry (MS) and Nuclear Magnetic Resonance (NMR) spectroscopy (Fig. 3 D). These approaches allow for the identification and enumeration of metabolites, involved in PGCs formation and differentiation. Various analytical techniques in metabolomics which might be useful in understanding the changes that occur in PGCs as they develop into somatic gametes (Rengaraj *et al.*, 2013)

Regulation dynamics of metabolism or metabolic profiles during PGCs development: The nutritional metabolism of PGCs also changes considerably during specification and migration. PGCs are moderately metabolically active and their activity peak is distinct during early stages of development. Glycolysis, the fuel for PGCs migration and proliferation. Fatty acid metabolism involved in development of cells' membranes, which defines cell function. Germline progenitor cell development, nucleotide metabolism anticipated in purine/pyrimidine biosynthesis to promote germ cell proliferation is hence critical.

Key metabolic pathways involved in PGCs development: Glycolysis pathway*:* this pathway can stimulate the immune system at the beginning of migration, as well as during the fate determination. This is beneficial to maintain the undifferentiated state of stem cells. Fatty acid metabolism:- has the greatest importance in metabolic processes within the cell and in signaling (Song et al., 2022) . Retention of amino acid metabolism:- this is crucial for bringing necessary proteins for energy production and synthesis of proteins important for metabolic equilibrium and important in the high proliferative cells like the PGCs. Nucleotide metabolism:- Act as stimuli for cell division and for the synthesis of deoxyribonucleic acid (DNA) (Rengaraj *et al.*, 2013; Marlow, 2015; Huang *et al.*, 2022). Oxidative Phosphorylation (OXPHOS):- play very important role in PGCs by energy production by producing ATPs, used for PGCs survival and proliferation (growth and differentiation) as well as migration. OXPHOS is involved in metabolic programming of PGCs by providing them ability to adopt changes, also regulates mitochondrial biogenesis and helps to maintain healthy mitochondria. (Fig. 3 A) (Hayashi *et al.*, 2018; Yuan *et al.*, 2021).

Influences of metabolite levels on PGCs: PGCs identity is under direct metabolic control so nutrients can either promote the development of PGCs, enhance their proliferation, or increase apoptosis (Yang *et al.*, 2022) . The availability of glucose inhibits PGCs survival whereas amino acid availability enhances germ cell function, so nutrients can influence PGCs differentiation. Carbohydrates are important for the fate determination of germ cells because a lower glucose level limits PGCs like cells formation (Hayashi and Matsui, 2022). Hexose amine biosynthetic pathway (HBP), a metabolic pathway for glycolysis results in (UDP-GlcNAc) uridine diphosphate N-acetylglucose-amine production promotes PGCs specification under glucose influences. A nutrient sensor protein named as (O-GlcNAcylation) O-linked b-Nacetylglucosaminylation, based on (UDP-GlcNAc)

controls signaling, transcription as well as epigenetics by post-transcriptional modifications. BLIMP1-mVenus a protein, PGCs markers controlling pluripotency and specification and PGCs-related mesodermal cells are influenced by depletion of glucose levels (Fig. 3 B) (Hayashi *et al.*, 2023). Each of the identified metabolic pathways is involved in the differentiation of chicken germ cells. Chick ciliary ganglia contain PGCs that have been studied using metabolomic techniques which have applications for the explanation of distinct metabolic states (Zhao *et al.*, 2023). Chicken studies related to PGCs and employing analytical metabolomics approaches provides information for survival, migration and differentiation of PGCs at diverse metabolic conditions, and metabolic diseases or experiences external stress (Peng *et al.*, 2018).

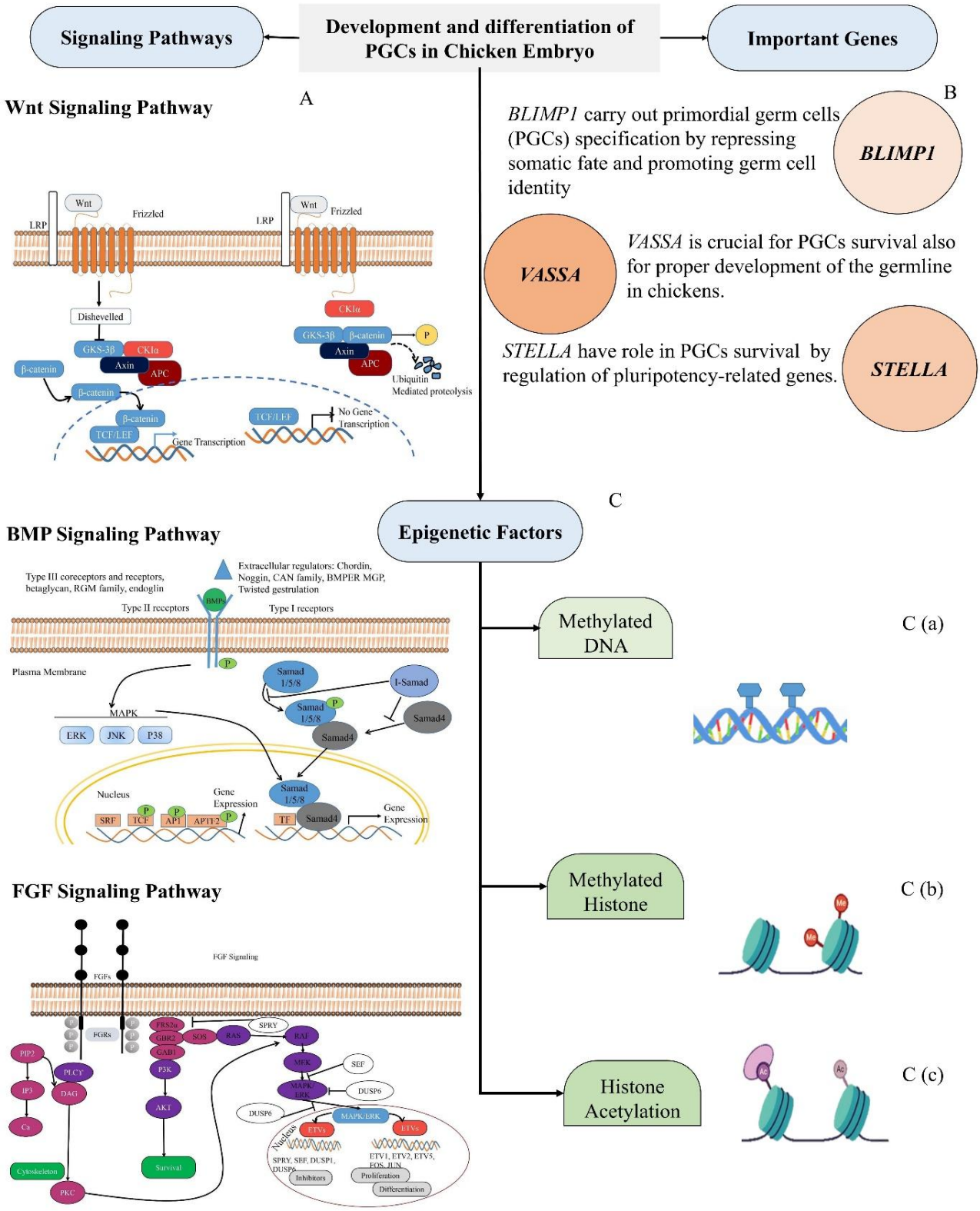

Fig. 2: Development and differentiation of embryonic PGCs in chicken. Note: A Some essential signaling pathways B. Important genes C. Molecular regulation C (a) shows DNA methylation, while C (b) and C (c) are showing histone methylation and acetylation respectively.

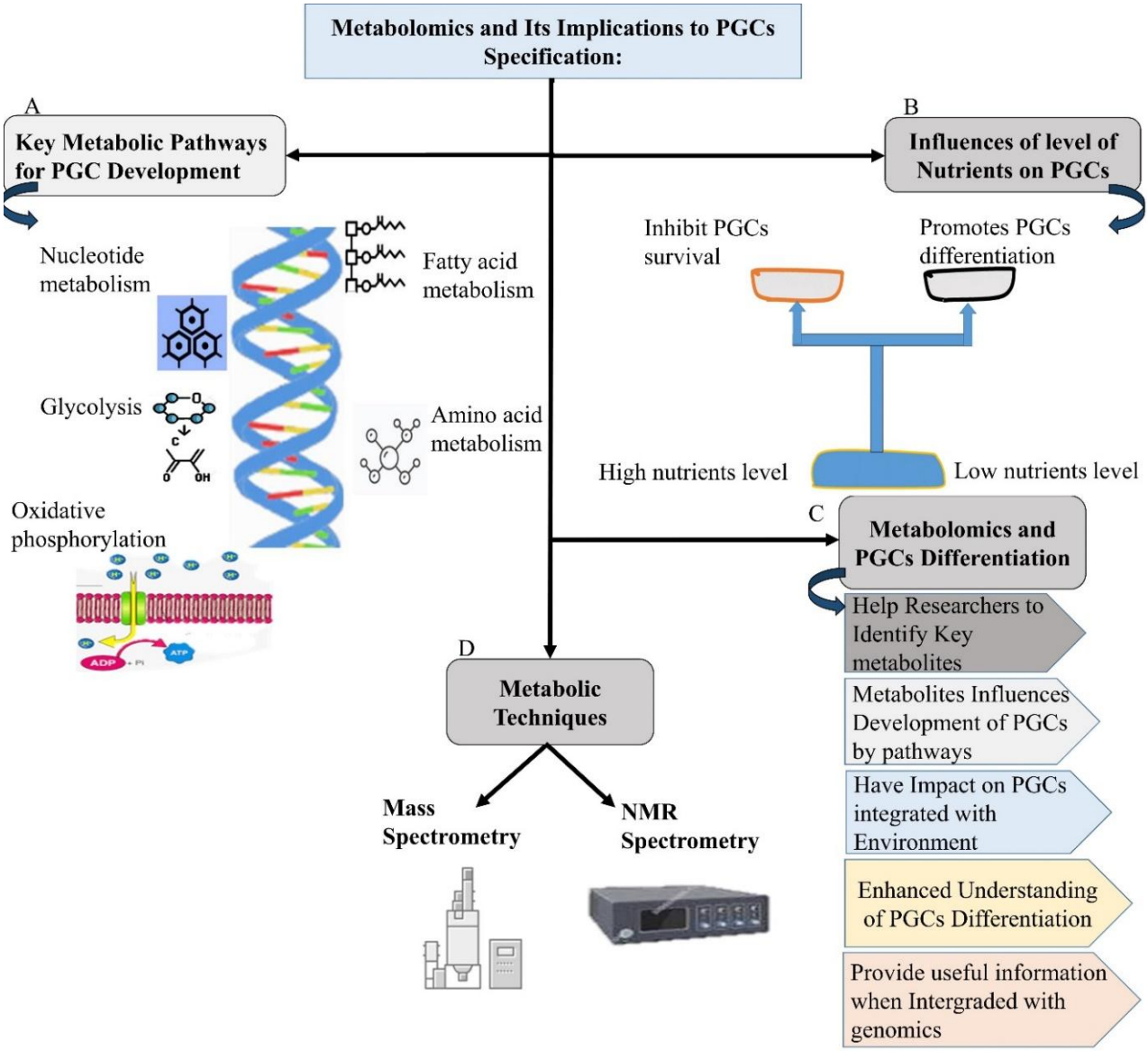

Fig. 3: Metabolomics and its implications in PGCs Specification. Note: A. Key metabolic pathways that are involved in controlling PGCs B. Nutrients levels influence on PGCs C. Different roles of metabolites and metabolomics study in PGCs regulation.

Metabolomics applications: The application of metabolomics in chicken PGCs is relatively recent. Metabolic profiling and identification of important metabolic pathways helps to elucidate the map through which chicken germ stem cells transition could be explained to a terminally differentiated germ cell state (Zhang *et al.*, 2016). Studying the pulmonary glossopharyngeal in chicken through metabolomics approaches help us to understand what is known about the effects of metabolic diseases and environmental stressors on germ cell (Fig. 3 C) (Hayashi and Matsui, 2022).

Genomics overview and its role in chicken PGCs: In PGCs molecular biology is one of the prominent factors that define the formation, specification, and differentiation of the PGCs in the chicken embryo at different stages of development from epiblast to gonads (Fig. 4 A). Molecular biology has a set of principles based on genetics and epigenetics. Advanced genomically and transcriptomically derived data have provided major insight into molecular pathways that regulate PGCs cell fate determination.

Genomics techniques: Genomics employs several coexisting complex techniques, some of the key techniques include: Next-Generation Sequencing:- technology is being used to study DNA sequence, all RNA transcripts and even to study changes in the chromosomes, gene expression schedules and data associated with development processes, including PGCs formation are easily discerned using NGS technologies. Genome-Wide Association Studies (GWAS):- GWAS is used to identify single nucleotide polymorphisms (SNP) related to some specific traits including Germ cell differentiation and sex determination. In chickens PGCs studies pointed out the use GWAS to identify the chromosomal regions harboring genes that regulate the production of PGCs and to help us to understand the mode through which genes in chickens predict the developmental potential of PGCs (Fig. 4 C) (Pértille *et al.*, 2016).

Genes with significant roles in PGCs formation: In chicken PGCs, many studies have identified key genes for PGCs specification, maintenance, and differentiation. Key

genes includes; *PRDM1 (BLIMP1)*: *BLIMP1* controls chicken PGC differentiation. It activates germ cell identity genes (Jang *et al.*, 2013). *NANOG* (Nanog homeobox):- *NANOG* is a pluripotency gene that makes stem cells renewable (Zhang *et al.*, 2021). Chicken embryonic stem cells (ESCs) also maintain pluripotency with *NANOG*. At Hamburger Hamilton stage 5 (HH5) to HH8, JAK1/STAT3 (Signal Transducer and Activator of Transcription 3) signaling supports chicken embryonic pluripotency and *NANOG* expression. ESCs and PGCs have different *NANOG* protein levels. This gene is specifically upregulated to promote PGCs development while supporting initial pluripotency (Jung *et al.*, 2018; Choi *et al.*, 2021). *OCT4* (Octamer-binding transcription factor 4):- Pluripotency factor *OCT4* is another *LIN28* isoform needed for PGCs stem cell identity and survival and controls DNA methyltransferases (Jang *et al.*, 2013). Other genes; *CXCR4* (C-X-C motif chemokine receptor 4) gene expression was high until 2.5 days, then decreased, resulting in fewer PGCs or gonadal germ cells GGCs in the gonads than in the blood. *SSEA*-1: Stage-Specific Embryonic Antigen 1 in male gonads with settled PGCs show decreased expression of gene and in female gonads, *SSEA*-1^{+} cells were constant but expression decreased. *SSEA1* cells are small in females because morphological differentiation is controlled by sex determinant genes on day 3.5 and day 6 (Motono *et al.*, 2008b,). The metabolism-genetics relationship is shown by the fact that *STELLA* gene expression did not change during glucose depletion but increased in 2 Deoxy Glucose-mediated glycolysis inhibition (Hayashi *et al.*, 2023)**.** At early stages, genes and regulatory networks control PGCs, and later, chicken gonads. *SMAD7* (SMAD family member 7) and *NCAM2* (Neural Cell Adhesion Molecule 2) gene overexpression in female cells may increase cell-cell adhesion and slow proliferation (Doddamani *et al.*, 2023).*VASA* and *STELLA*: Germ cell markers that maintain pre-GCZ germ cell status. *SOX2, SRY* (sex determining region Y-box 2), another transcription factor, is also needed to maintain germline cell pluripotency during development (Fig. 4 B) (Mathan *et al.*, 2023).

Genomics significance for chicken PGCs: Germline stem cell differentiation in chickens is controlled by some specific genes and pathways (Ichikawa and Horiuchi, 2023). Signaling pathways in PGCs specification including WNT, BMPs, and FGF signaling pathways. Epigenetic factors are important, it is proved that the histone methylation (H3K4me2/Histone H3 Lysin4 Demethylation) is required for generation of chicken PGCs (Fig. 4 D) (Zhang *et al.*, 2021). Ordered epigenetic methylation regulates genes that are important for PGCs specification and shows that in early germ cells stages, chromatin remodeling occurs.

CRISPR/Cas9 and functional genomics in PGCs research: CRISPR/Cas 9 method of gene editing a very useful to PGCs in the present work functional genomics is also proved useful. It allows a precise change or editing in genes linked with PGCs formation to regulation (Han and Park, 2018). For example, use of CRISPR/Cas9 to create chickens that carry knocked-in genes used to investigate PGCs fate and sex determination potential (Hagihara *et al.*, 2020). This makes it easier for the researchers to investigate the molecular regulation of PGCs formation and maintenance in a mechano-specific method (Soler *et al.*, 2021). Also used for other avian species like in the study researcher managed to obtain genetically modified quails by directly injecting CRISPR/Cas complex into injected quail zygotes (Fig. 4 Ca) (Mizushima *et al.*, 2023).

Genetic markers in PGCs:- By fluorescein dye-labeled PGCs in embryos were infected with leukosis virus, for identification and validation of specific genetic markers for PGCs. Scientist concluded that in PGCs, there are genetic markers identified as *VASSA*, *STELLA, BLIMP1*(Jang *et al.*, 2013). These can be employed in developmental studies, to detect and separate PGCs during subsequent examinations or even in germline manipulation (Zhang *et al.*, 2015b).

Transcriptional and epigenetics role in PGCs regulation: Epigenetic regulation plays a critical role in both the formation and maintenance of the germ cell lines specifically PGCs. Changes in histones, and DNA methylation patterns are required for the regulation of PGCs specific genes such as *NANOG*, and *BLIMP1*. For example study has shown that DNA methylation contributes to the post-implantation paternal genome of PGCs (Jang *et al.*, 2013). Epigenetic programming of chicken germ cells help at significant level in the transcriptional regulation or chromatin modifications. This is necessary in the downregulation of somatic genes and upregulation of germline genes or pathways (He *et al.*, 2018). Epigenetic factors are stable and reduced after birth, while DNA methylation and its changes in developing embryos are tissue- and time-dependent (Gryzinska *et al.*, 2013; Li *et al.*, 2015). Genomics helps in identification of *BLIMP1, NANOG* and *OCT4* and the genes controlling histone at its level including other histone methylation H3K4me2 (Fig. 4 D). (Kumar *et al.*, 2020).

Transcriptomics and its role in chicken PGCs:

Overview of transcriptomic: Transcriptomics is stage and time specific moreover every breed of chicken has a different transcriptomic profile and can differ at different embryonic development stages. Epigenetics regulate gene expression during embryonic development, which is methylation-dependent (Dunislawska *et al.*, 2021). Epigenetic modifications methylation on H3K4me2 and lncCpSET1 upregulate formation of PGCs. Gene ontology (GO) chromatin immunoprecipitation sequencing (CHIP-seq), functional annotation, heat map analysis Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis, CHIP online database analysis accompanied with qRT-PCR resulted in, lncCpSET1 and H3K4me2 regulating *FZD2, ID1, ID4,* and *BMP4* genes (Fig. 5 A). Immunoprecipitations of RNA (RIP) and (CO-IP) co-immunoprecipitation results indicated lncCPSET1 increase *BMP4* expression and promote PGCs formation by binding DPY30 protein (Ding *et al.*, 2024). RNA isolated for transcriptome analysis for gene expression changes at day 4.5, PGCs number were increased during intense proliferation until day 5-to-8-day somatic cells started increasing and 12-day somatic conversion. Embryo sex affects growth time. High gene regulation on days 8–12 indicates intense biological activity. Using transcriptome study, *SPARC* (Secreted Protein Acidic and Rich in Cysteine) gene was upregulated, *NIS* (Sodium Iodide

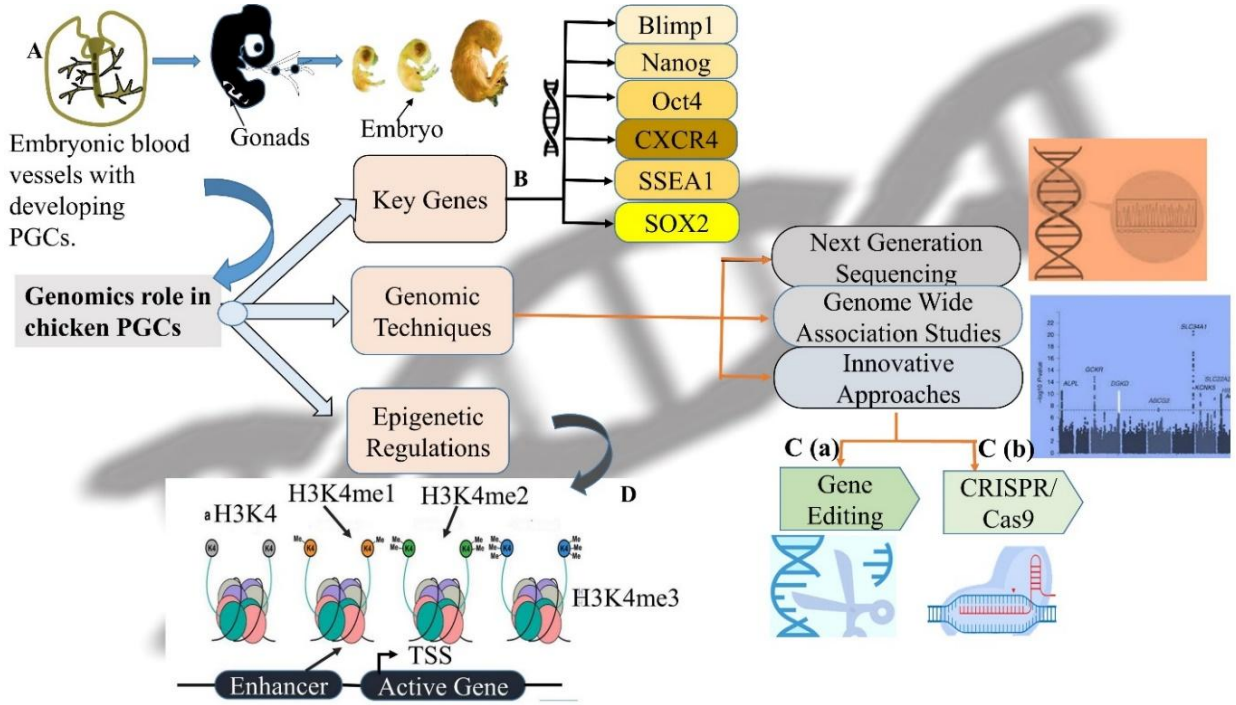

Fig. 4: Genomics role in Chicken PGCs. Note: A. Is showing different stages of embryonic PGCs development from blood vessels to mature embryo, influenced by genomics. B. Key genes. C. Genomic techniques. D. Epigenetic regulation of genes, or genes reprogramming, how H3K4me2 methylation and active gene.

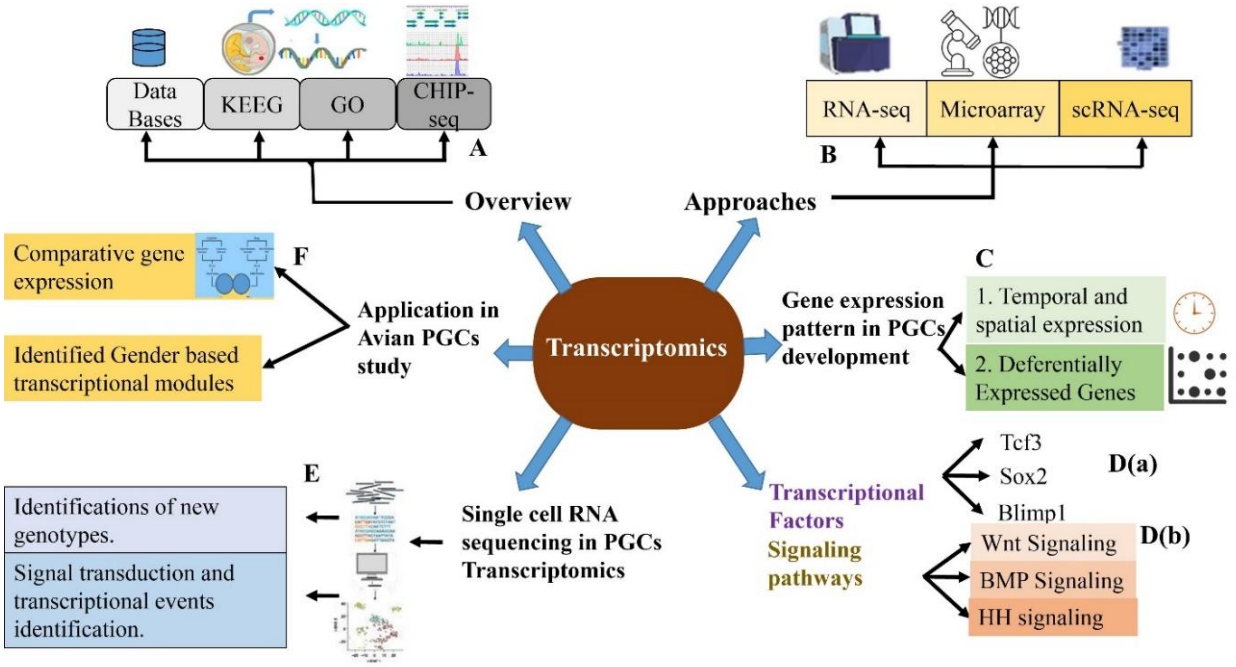

Fig. 5: Transcriptomics overview and its role in PGCs. Note: A. This part is showing general transcriptomics tools and data bases to generate and interpret transcriptomic data to find out biological procedures and genes regulating PGCs at that time. B. This is showing RNA sequencing techniques can be used in PGCs to find out genes. C. Expression patterns of gene. D. Transcriptional factors and signaling pathways involved in PGCs formation differentiation & migration. E. Is elaborating role of scRNA sequencing in PGCs. F. Application of transcriptomics in Avian PGCs.

Symporter) gene and *PERPINB10* downregulated on day 8 and increased on day 12 (Dunislawska *et al.*, 2020a). RNA-seq and Gene Expression profiling in chicken PGCs have proved an important contribution towards determining the molecular mechanisms of formation, specification, and differentiation of PGCs in chickens. Microarray or RNA-seq can be used to study gene expression changes during PGCs development, and scRNA-seq can be used to visualize data single cell level (Jovic *et al.*, 2022).

Transcriptomics approaches: Transcriptomics described as the analysis of large amounts of RNA that the genome produces in their response to given situations. It provides information on gene expression and can reveal significant regulative pathways and genes relating to its formation. Main techniques used in transcriptomic research include RNA sequencing, Microarray, single cell RNA sequencing (Lowe *et al.*, 2017). RNA Sequencing (RNA-seq): RNA-seq is one of the most used methods to identify RNA

expressions, which form a basis for further investigations of gens expressions, RNA-seq is most valuable in identifying Differentially Expressed Genes (DEGs) in specific cell types, while PGCs in development. This method is very specific and suitable for obtaining low abundance RNA species (Zhang *et al.*, 2015a). Microarrays: Although microarrays much popular as RNA-seq, the technique is used in several experiments to analyze gene expression under many different circumstances. Microarray technology may be used to quantify the signal strength of the genes which have been identified and may be used to study differential gene expressions in the formation of PGCs (Dunislawska *et al.*, 2021). Single-Cell RNA sequencing (scRNA-seq): is a technique to perform analysis of genes at single cell level and is used to study cell heterogeneity, Lineage, determination and gene expression of in specific cell type. This technology has therefore been useful in demonstrating the existing heterogeneity in PGCs and the early lineage programs in which PGCs are directed away from gonadal tissues. In chickens, the molecular profiles of gonadal PGCs are described in detail by scRNA-seq (Fig. 5 B) (Jovic *et al.*, 2022; Jung *et al*., 2023).

PGCs development and gene expression patterns: Temporal gene expression profiles of PGCs during development, clear pivotal shifts in the signaling pathways that regulate cell fate determination. The effects are altered by the alteration in the mode of gene codes to the gonadal surrounding as already mentioned in studies (Dunislawska *et al.*, 2020b; Jin *et al.*, 2020). For example, *VASA* and *STELLA* gene, which are germ cells related genes, are active only during the specification phase of the germ cells development. While *SOX2* that defines a factor of pluripotent stem cells remains active in the migration and gonadal stages of germ cells development. Through single cell gene expression analysis in developing mice demonstrated that germline specification is associated with the dynamical alterations in the gene network that controls specific cell fate decisions. These can be analyzed in chickens also (Lu *et al.*, 2024). Differentially expressed genes: DEGs are significant at some specific stages of chicken PGCs development. It is identified that these genes help to generate important functions of cellular communication, cell migration ability and cell survival of most cells in the organism. These results suggest that relative levels of these genes indicate that PGCs development relates solely to both intrinsic transcriptional and extrinsic provisions (Fig. 5 C) (Doddamani *et al.*, 2023).

The transcriptional factors and signaling pathways: Several key pathways and transcription factors have been identified as crucial for PGCs development including. Transcriptional regulators: *SOX2:* Which is a system that help to sustain the function of the cell to remain stem like. Another component with which it collaborates to regulate the ratio between proliferation and differentiation is Tcf3 a protein. Responsible for regulation of Wnt signaling pathway and is involved in the determination and development of PGCs. *BLIMP1:- BLIMP1* is a master factor that is critical for specification of PGCs this is because of two reasons, first, it represses somatic genes repressing PGCs specification and, second, it activates germ cell-specific genes (Fig. 5 D) (Naeemipour *et al.*, 2013). Signaling Pathways: BMP Signaling: Another important signaling pathway that regulates PGCs fate is the BMP or Bone Morphogenetic Protein pathway. It controls genes that define the fate of PGCs and their subsequent differentiation; the genes include *SOX2* and *BLIMP1* (Suzuki, 2023). Hamburger Hamilton HH signaling: is necessary for adequate PGCs colonization of the gonadal region HH signaling regulates the transcription of proteins for stem cell differentiation into PGCs (Fig. 5 D) (Zare *et al.*, 2023).

Single cell RNA sequencing in PGCs transcriptomic study: The current scRNA-seq analyses of chicken PGCs showed high developmental stage heterogeneity. Using scRNA-seq, gonadal PGCs from zebra finches and chickens were analyzed, revealing new genotypes that define PGCs development (Jung *et al*., 2023). Sexually dimorphic genes were found in chicken migratory PGCs by scRNA-seq. Explained male and female PGCs gene resources (Fig. 5 E) (Zou *et al.*, 2025).

Application of transcriptomic methods in avian PGCs research: RNA sequencing in chicken PGCs and ESCs revealed different transcriptional modules or factors responsible for male and female PGCs, which are important for fate determination and germline development (Jin *et al.*, 2020). Using the proteomics approach researchers examined the dynamics of events. The male and female PGCs have unique transcriptional signatures that explain sex determination and germline development (Fig. 5 F) (Zuo *et al.*, 2023a). RNA-seq and scRNA-seq are novel methods for measuring chicken PGCs differentiation. Hence, spatial comparison at different stages, DEG identification during PGCs development, transcription factors, and signalling pathways provide more PGCs specification information. Further scRNA-seq helps study germ cell development's genetic and epigenetic regulation and breeding and reproduction (Jax *et al*., 2018; Magar *et al.*, 2022).

Proteomics in PGCs formation and regulation of their development: Proteomics is the study and identification of proteins' functions. Mass Spectrometry, 2D-gel electrophoresis, and label-free quantification can reveal how PGCs transcription factors and signalling pathways regulate protein expression and post-translational modifications during specification, migration, and cell fate determination. Researchers can check the role of proteomics in chicken PGCs and compare proteomics at different developmental stages (Gong *et al.*, 2024).

Technologies of proteomics and uses: Technologies involve in the identification of proteins and their roles in the cells and most widely used proteomic methods in PGCs research involves, Mass spectrometry (MS): this is a suitable method for protein identification and quantification in samples of biological fluids. The peptides are ionized, and the mass to charge ratio is calculated, making it possible for MS to identify proteins, including those from the PGCs, which are a low abundance population. MS is usually combined with techniques

including LC (LC–MS/MS) that enhances the degree of identification of proteins(Wu *et al.*, 2009). 2Diamentional-Gel Electrophoresis: 2D-gel electrophoresis separates proteins based on two properties, desirable pH and molecular weight. Despite a lower sensitivity of this method as compared to MS for the identification of novel proteins. Its more useful for the comparison of relative changes in the expression of proteins during PGCs differentiation at different stages (Alessandroni *et al*., 2024). Label-Free Quantification: Quantification by intensity comparison and does not require labelling the proteins are quantified with respect to the number of ions identified in the MS data set. By using this technique, the absolute quantification of temporal changes of proteins in the developing stages of PGCs are achievable without IASL (Fig. 6 A)(Aslam *et al.*, 2016).

Recognition of proteins in chicken PGCs: Some of the new molecular findings identified by the proteomics method include proteins that participate to the generation, movement and differentiation of chicken PGCs. These proteins also have roles in the differentiation of the germline layer, protection of germ cell lineage and influence on migration of PGCs to the gonads (Kunec and Burgess, 2015). Key proteins involved in chicken PGCs regulation includes VASA: An RNA helicase specific for the germline cells, involved in PGCs destiny, Vasa is also important molecular component of PGCs and is related to the function in RNA metabolism and germ cells development. DAZL: RNA-binding protein that promotes activity and replication of germ cells by modulating the translation of target mRNAs (Lee *et al*., 2016). SOX2: is able to establish a complex with other molecules in order to control the destiny of PGCs. TDRD7: It is important protein that is involved in germline protection in model organisms and seems to have epigenetic roles in spermatogenesis and is associated with both piRNAs and the repression of transposons (Ferver *et al.*, 2022). Using MS with 2D gel electrophoresis, indicated that these proteins were up regulated in early PGCs. For example VASA protein was identified in several PGCs from their formation up to migration period. However, DAZL and TDRD7 as well as seems to play a critical role in PGCs survival and retention of their identity before the gonad development period (Fig. 6 B) (Zuo *et al.*, 2023a).

PGCs development and proteomic profile: Proteomics approaches have been used to identify temporally exclusive sets of proteins expressed during PGCs development. That helps in understanding of the developmental versatility during the PGCs specification, migration and differentiation. Early development, PGCs specification proteins: BLIMP1 and NANOG as transcription factors, are uniquely found in early stages of PGCs specification.The protein are crucial for preserving the pluripotency and protecting against somatic commitment in PGCs precursors. Migration Phase: PGCs start destroying at a point, so cells migrate towards the gonads with the help of proteins like migration-inductive proteins like CXCL12/SDF1, which promote PGCs movement in a direction with a chemical gradient. During this phase, the proteomic profile includes proteins involved in cell adhesion and motility, or cytoskeleton (Zuo *et al.*, 2023a). Gonadal Entry and Differentiation: Finally, after entering gonads, PGCs begin to differentiate intensively, changing the spectra of proteins to the genes that are linked with entry to meiosis and sex determination. Some proteins are clearly shown up-regulated during the period of the PGCs entry to meiosis or any kind of cell decision making, such proteins are Stra8 and GDF9.

The proteins participate in fate determination of PGCs: PGCs development is regulated by many germ cell fate-related proteins. PGCs fate determination proteins include VASA: This protein allow the germline RNA translational regulation and stabilization in PGCs, and thus to remain pluripotent and to perform self-renewal. DAZL (Deleted in Azoospermia-like): This RNA-binding protein control the germ cell survival and proliferation to synthesize the initial form genes necessary for the early phase of PGCs**.** TDRD7:- Play epigenetic role as a regulatory protein, TDRD7 (Tudor Domain Containing Protein7) interacting with piRNA to mitigate TE activity in germline responding to epigenetic disruption and maintain genome integrity in PGCs. But the proteins such as BMP4 and Wnt are selective in the manner they help PGCs to differentiate, they can also play a role by PPI (protein-protein interaction). BMP4 signalling is shown to control this process from pluripotency to differentiation while Wnt signaling for the balance of self-renewal and differentiation of PGCs. This family of proteins regulate the gonocyte and spermatogonial stem cell differentiation and similar process to that observed in chicken PGCs (Fig. 6 C) (Han *et al.*, 2005).

Integrated OMICS in chicken PGCs as well as in poultry reproduction: When proteomic data is combined with genomic and transcriptomic data, PGCs regulation becomes clear. Genomic studies genes and mutations, transcriptomics studies gene expression, and proteomics studies cellular proteins. (Dehau *et al.*, 2022). So total data can lead to solid results, but the major drawback is a large amount of data.

Application of the 'multi-omics' technologies in the study of PGCs: Genomics and Proteomics: Genomics provides us with information on which genes have the potential to contribute to fate determination of PGCs. Proteomics informs which of these genes are translated into protein in the organism (Zuo *et al.*, 2023a).

Transcriptomics and Proteomics: Transcriptomic the expression of genes and proteomics is the product of expression when integrated help us to identify certain instances or situation, where mRNA levels differ from protein levels might indicate post-transcription regulation processes, or Protein post-translational modifications (Ferver *et al.*, 2022). Disturbance in these models of quantitative and qualitative systems can disturb understanding PGCs development through the genomic, transcriptomic and proteomic interactions (Jin *et al.*, 2020). Together with the proteomics data, the ever expanding genomic and transcriptomics data provide a wide perspective on developmental signaling and the molecular processes of PGCs formation (Kumar and Gupta, 2024). When protein profiles are generated at different developmental stages of PGCs it make it feasible to identify

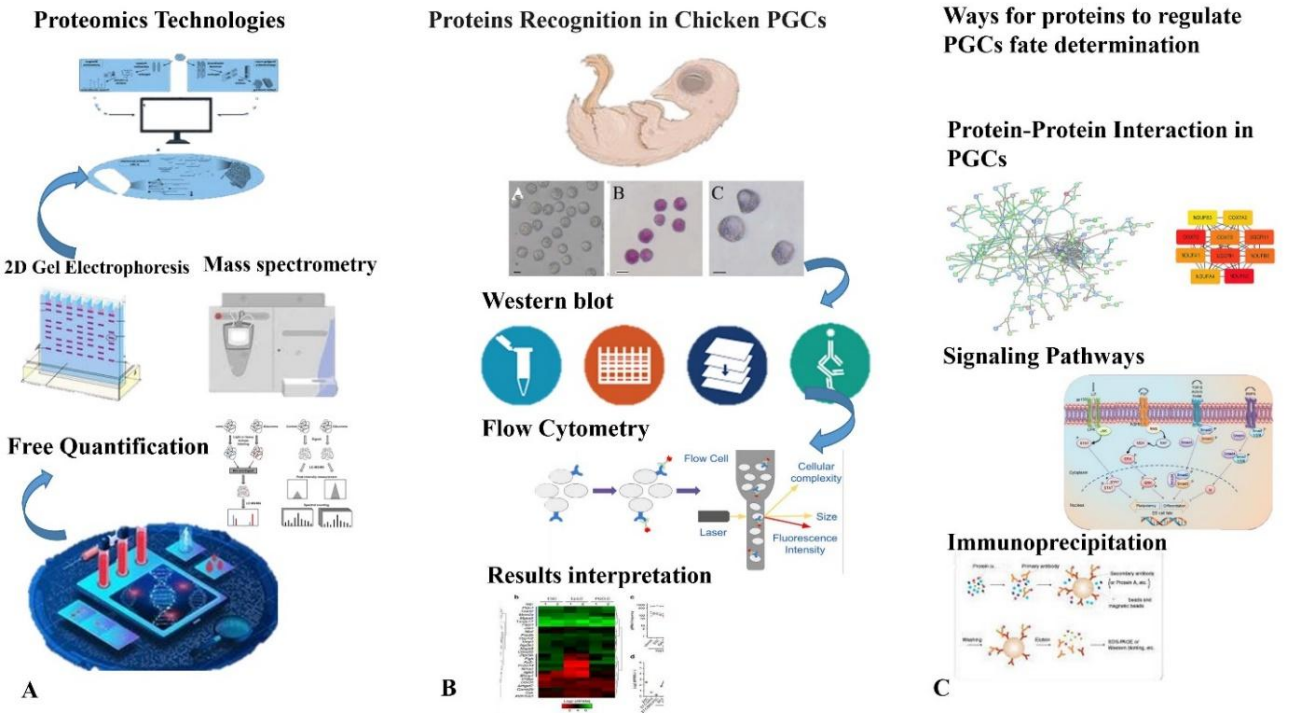

Fig. 1: Proteomics and role in chicken PGCs. Note: A. Proteomics technologies B. Process of protein recognition in chicken PGCs C. the ways by which proteins can control fate determination of PGCs.

prime proteins and signals that are essential in germ cells development, movement and differentiation. Multiplex methodologies, currently referred to 'MULTI-OMICS' are developing significantly and helping to define very fine and complex molecular signaling networks that result in determining developmental programs, and continue to be essential resources for those who study germ cells in the future (Zuo *et al.*, 2023b).

Integration various 'OMICS' for poultry breeding:

Poultry genetic resources improvement by OMICS: Genomics, transcriptomics, proteomics, and metabolomics are four parts of OMICS, these can greatly contribute to genetic resources improvement in poultry breeding because other to determining the genes that are responsible for reproductive success like fertility traits, the technologies also provide information on which biological processes are functioning (Wadood and Zhang, 2024). These technologies can help in identifying diseases, control or enhance chicken growth as well as reproduction , can also support improved selection (Selvam *et al*., 2019).

Genomics and poultry breeding: The current genomics data can help to find genes that are associated with reproductive traits like egg production, fertility, and hatchability. Along with the specific parts of GWAS, transcriptomics, and proteomics data, it assists in the prediction and selection of positive genetic gain components related to reproduction. Thus, QTL mapping also plays an important role in the regulation of source-sink transition and other inherited traits concerning reproductive health (Shen *et al.*, 2024)

Germline Modification: OMICS technologies also help in the molecular assessment of germline structures of birds which are essential in improving reproductive productivity. Egg quality, and conception probabilities could be directly improved through the genetic modification of genes elicited to fertility (Dehdilani *et al.*, 2023). Understanding of the germline genes through transcriptomics is useful for germline genetically improved lines of poultry (Challagulla *et al.*, 2023).

Therapeutic applications and germ cell transplant: OMICS technologies applied in Stem cell therapy and cell-based reproductive technologies, also to improve the fertility (Qiao *et al.*, 2024). Due to Gene Ontology accessibility, transcriptomic and proteomic studies can be applied to define the determinants highly involved in PGCs development and differentiation (Nakamura, 2017). OMICS concerned with germ cell transplantation and PGCs are being analyzed in a particular developmental stage, the analysis of gene expression at the single cell level has become particularly relevant by use of scRNA-seq (Liu *et al.*, 2022). Specific signaling pathways could be identified and better germ cell mediated reproductive technologies could be used most relevant to germ cell transplantation. (Suravajhala *et al*., 2016).

Here we would like to assemble all the information in a vast summary of effect OMICS on chicken PGCs and other uses. When PGCs start to develop in the genital ridges regulations of processes of their formation, fate determination as well as migration are very important for successful reproduction system development. They need instructions in the form of genomics data that we have described role of different important gene. Genomics leads towards transcriptome formation transcriptomes are all types of RNAs and control PGCs. Transcriptomics could be used to create complementary DNA and by making complementary DNA we can find parent genes regulating the PGCs at specific stages of development at specific times, and regulatory pathways. We can use proteomics profiles to determine how many active genes make proteins

and biological molecules. Metabolomics can identify nutrients and metabolic pathways that regulate PGCs at different stages. In these OMICS technologies, Gene Ontology and KEGG are very helpful for researchers to co-relate or find out pathways regulating genes. These technologies in these ways are helping and promising to help in molecular biology and reproduction of chicken. By integrating knowledge of nutrients, genes, proteins, and regulatory pathways, researchers could be able to control PGCs formation, differentiation and migration processes and ensure high production of PGCs ultimately resulting in improved reproductive output (Sasanami, 2017). Because these studies can identify markers and genes of traits of interest, OMICS data can aid CRISPR/Cas9 and other gene editing technologies. OMICS approaches in poultry with GWAS and QTL mapping can improve reproduction by identifying genes related to fertility, ovaries, and reproductive diseases, which can improve poultry bird selection. It can also aid germline transplant for poultry genetic disorders.

Future prospective: PGCs can be culture and utilized for gene editing at very early stage of next generations life for producing the transgenic chickens with high accuracy. OMICS can identify PGCs related genes, signaling pathways, and epigenetic regulators, which can be used to increase PGCs production. Increased PGCs production can produce a large number of offspring, a reservoir of endangered or important breeds. The use of PGCs as stem cell therapy by replacing germ cells at PGC stage has many research gaps. Recent advances in gene editing like CRISPR/Cas9 system and single cell RNA sequencing are helping to understand avian PGCs and allow gene editing in PGCs, enabling complicated, better-controlled poultry manipulations. Additionally, long-term PGCs culture models from embryonic or adult sources provide development and differentiation data. That aids reproductive science, germ cell culturing, and poultry transplants for reservoirs. OMICs can also improve poultry fertility, hatchability, and reproductive efficiency mechanisms. Epigenetics is crucial to PGCs molecular regulation, and epigenetic reprogramming is unknown, promising researchers a place in molecular biology.

Conclusion: In conclusion we can say several recent studies of integrated with metabolomics, or individual genomic, transcriptomic, and proteomic analyses have contributed and increased current understanding of chicken PGCs formation and regulation mechanisms. The 'OMICS' technologies have given knowledge about general aspects like gene and biomarkers connected with PGCs development and regulation, which is enhancing the reproductive biology. Innovative technologies like OMICS approaches, when used for poultry at PGCs level provides us information about factors that are controlling germ-cell differentiation. By using transcription activator-like effector nuclease (TALEN) and CRISPR/Cas9 tool for knocking down and interfering in the function of these genes or making new possibilities in reproductive biotechnology or germline engineering most precisely.

If we focus on improvements of avian reproductive biology, when we integrate functional genomics with transcriptomics and epigenomics their integrated results seem to be helpful for PGCs development and avian breeding and conservation in the future. Poultry 'OMICS' studies are not only extremely useful and crucial in the improvement of reproductive biology of poultry but also have a role on other birds as well. There is one major drawback of OMICS-based studies in poultry reproduction, the big MULTI-OMICS data analysis, costs, and concerns over active genome modification, but this study is still very promising. This is a slow-growing field and still has many opportunities for the further development of PGCs research.

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