

BURLEIGH DODDS SERIES IN AGRICULTURAL SCIENCE

Embryo development and hatchery practice in poultry production

Edited by Dr Nick French



Contents

Series list	xi
Introduction	xxi

Part 1 Parental influences and embryo development

1	Genetic selection to improve reproductive traits in chickens	3
	<i>David Caverio and Maurice Raccoursier, H&N International, Germany</i>	
1	Introduction	3
2	Layer breeding programme structure	4
3	Egg production	7
4	Liveability	11
5	Resilience and adaptability	12
6	Egg quality	13
7	Nesting behaviour	18
8	Reproduction traits	19
9	Hatchability	21
10	Fertility	22
11	Sperm quality	24
12	Molecular tools	26
13	Future trends	26
14	Conclusion	27
15	Where to look for further information	28
16	References	29
2	Managing breeder poultry flocks to optimise hatchability and chick health	35
	<i>R. A. van Emous, Wageningen Livestock Research, The Netherlands</i>	
1	Introduction	35
2	Mating behaviour	36
3	Housing system	38
4	Male-to-female ratio	42
5	Spiking	45
6	Nutrition	47

7	Feed restriction and feeding programs	53
8	Body weight for females and males	54
9	Feather cover	55
10	Where to look for further information	56
11	References	56
3	Advances in understanding the development of defences against pathogens in the chicken egg <i>Maureen M. Bain, University of Glasgow, UK</i>	69
1	Introduction	69
2	Basic components of the egg	70
3	Formation of the egg: key events	71
4	Antimicrobial molecules associated with the egg and their mechanisms of action	74
5	Contribution made by each component of the egg to the egg's defence	74
6	Embryo-derived structures involved in the egg's defence	80
7	Conclusion	80
8	Where to look for further information	81
9	References	81
4	Assessing poultry semen quality <i>Murray R. Bakst, formerly USDA-ARS, USA</i>	87
1	Introduction	87
2	Biology of fertilization	89
3	Evaluation of semen	92
4	Fertility determination	95
5	Conclusion	96
6	Acknowledgements	97
7	Appendix	97
8	References	97
5	Key stages of embryo development in poultry <i>Marleen Boerjan, formerly R&D Royal PasReform, The Netherlands</i>	101
1	Introduction	101
2	Nomenclature describing the different phases of embryonic development	102
3	Fertilization: polyspermy and formation of the one-cell embryo/zygote	103
4	Intra-uterine development: from zygote to Eyal-Giladi stage X at oviposition	105
5	Embryo development during incubation	109
6	Endocrine control systems and maternal thyroids	116
7	Growth and development of the embryo E10-E15	117
8	E16-E19 plateau phase: the embryo shows endothermic responses	118
9	Extra-embryonic fluids and compartment development and function	119
10	Hatching	124

11	Conclusion	126
12	Acknowledgements	127
13	Where to look for further information	127
14	References	128
15	Appendix: Table 1 Chronology of the development of the chicken embryo	134
6	Chicken egg storage and transport <i>Dinah Nicholson, Aviagen Ltd, UK</i>	141
1	Introduction	141
2	Changes to the blastoderm during egg storage	143
3	Changes to egg quality during storage	145
4	Improving the survival of embryos in stored eggs	148
5	Turning eggs during storage	150
6	Transport	152
7	Egg shell contamination and disinfection	153
8	Short periods of incubation during egg storage	154
9	Acknowledgements	162
10	Conclusion	163
11	Where to look for further information	163
12	References	163
7	In ovo sexing in poultry chicks <i>Anke Förster, Agri Advanced Technologies GmbH, Germany; and Thomas Bartels, Institute of Animal Welfare and Animal Husbandry, Germany</i>	169
1	Introduction	169
2	Key issues and challenges	170
3	Case studies	174
4	Future research	181
5	Discussion	189
6	Conclusion and future trends	190
7	Where to look for further information	192
8	References	192
8	In ovo vaccination of chicken eggs <i>Christopher J. Williams, Consultant, USA</i>	197
1	Introduction	197
2	History and evolution of in ovo vaccination	198
3	Interacting factors of in ovo vaccination success	200
4	Additional interactions and considerations for in ovo vaccination	223
5	Conclusion and future trends	226
6	Where to look for further information	227
7	References	227

Part 2 Incubation

9	Incubator design for poultry eggs: principles and techniques <i>Ron Meijerhof, Poultry Performance Plus, The Netherlands</i>	233
1	Introduction	233
2	Temperature, heat production and heat transfer	234
3	Positioning and turning of eggs	237
4	Ventilation: managing moisture, CO ₂ and O ₂	238
5	Ventilation: managing heat	241
6	Cooling techniques	242
7	Incubator machine design issues	244
8	Hatcher design issues	246
9	Managing the hatching process	247
10	What should a hatcher look like?	251
11	Where to look for further information	252
12	References	252
10	Understanding the effects of incubator temperature on embryo and post-hatch chick development <i>R. Michael Hulet, Penn State University, USA; and Michael J. Wineland, North Carolina State University, USA</i>	255
1	Introduction	255
2	Single-stage versus multiple-stage incubation	256
3	Embryonic heat requirements	257
4	Effect of temperatures on hatchling quality and growth	259
5	Incubational thermal manipulation	261
6	Influence of embryonic temperature on organ development	262
7	Incubation temperature effects on incidence of ascites	268
8	Temperature effects on integument system	269
9	Conclusion	270
10	Where to look for further information	270
11	References	270
11	Understanding the effects of light on embryo and post-hatch chick development <i>Israel Rozenboim, Liron Dishon, Natalie Avital-Cohen and Joanna Bartman, The Hebrew University of Jerusalem, Israel; and Ronen Shviki, BeyondEdge Inc, USA</i>	279
1	Introduction	279
2	The role of monochromatic light on growth of meat-type birds	280
3	The effect of in ovo green light photostimulation on somatotrophic axis activity in embryos and post-hatch	288
4	Conclusion	297
5	References	298

12	Understanding the effects of humidity/air composition on embryo and post-hatch chick development <i>E. David Peebles, Mississippi State University, USA</i>	303
1	Introduction	303
2	Physical function of the eggshell as a respiratory organ for the developing avian embryo	304
3	Humidity	308
4	Vital gases	313
5	Conclusion	321
6	Where to look for further information	322
7	References	322
13	The role of egg turning in embryo development <i>Okan Elibol, Ankara University, Turkey</i>	331
1	Introduction	331
2	Turning during storage	332
3	Critical periods during incubation	333
4	Turning frequency during incubation	335
5	Turning angle during incubation	337
6	Signs of turning failure	338
7	Conclusion	338
8	References	339
14	Poultry embryo development and skeletal growth <i>Edgar Orlando Oviedo-Rondón, North Carolina State University, USA</i>	343
1	Introduction	343
2	Embryo bone development	345
3	Incubation factors that affect skeletal development	350
4	Conclusion	362
5	Acknowledgements	363
6	Where to look for further information	363
7	References	363

Part 3 Managing the hatching stage

15	Managing the poultry hatcher environment <i>Roos Molenaar and Henry van den Brand, Wageningen University & Research, The Netherlands</i>	375
1	Introduction	375
2	Embryonic phase	376
3	Environmental settings within the hatching phase	381
4	Conclusion	391
5	Where to look for further information	391
6	References	392

16	Alternative hatching systems for broilers <i>Henry van den Brand, Bas Kemp and Roos Molenaar, Wageningen University and Research, The Netherlands</i>	401
1	Introduction	401
2	Current alternative hatching systems	402
3	Differences between conventional and alternative hatching systems: environmental factors	403
4	Differences between conventional and alternative hatching systems: management factors	410
5	Effects of alternative hatching systems on health, welfare and production	414
6	Conclusion	419
7	References	419
17	Key issues in transportation of broiler and layer chicks <i>M. A. Mitchell, Scotland's Rural College (SRUC), UK</i>	425
1	Introduction	425
2	Transportation of newly hatched chicks	426
3	Welfare issues affecting newly hatched chicks: pre-transport	428
4	Welfare issues relating to transport of newly hatched chicks	432
5	Assessing welfare of newly hatched chicks	434
6	Journey times and periods without feed and water	434
7	Thermal stress in transit	440
8	Stocking density	449
9	Exposure of chicks to acceleration and vibration	451
10	Transport pathologies	452
11	Guidance on good practice	453
12	Conclusion	455
13	Where to look for further information	457
14	References	458
	Index	469

Introduction

It is widely recognised that developments in the chick embryo have a profound effect on hatchability and the subsequent health and productive capacity of poultry. With a stronger focus on welfare, prevention of disease and more sustainable poultry production, understanding these early stages has never been more crucial. This volume addresses the key stages and factors in embryo development in poultry to optimise hatchability and chick health.

The book is split into three parts: chapters in Part 1 examine parental influences and embryo development, focusing on areas such as the use of genetic selection to improve reproductive traits and management of breeder poultry flocks to optimise hatchability and chick health. Chapters on the development of defences against pathogens in the chicken egg, assessing poultry semen quality, the key stages of embryo development, chicken egg storage and transport, in ovo sexing in poultry chicks and in ovo vaccination of chicken eggs are also included. Part 2 chapters focus on incubation, specifically drawing attention to incubator design, the effects of incubator temperature, light as well as humidity and air composition on chick development. Chapters also address the role of egg turning in embryo development as well as the importance of incubation conditions on embryo skeletal development. Chapters in Part 3 focus on managing the hatching stage, highlighting the use of alternative hatching systems, the importance of managing the poultry hatcher environment as well as emphasising current key issues in transportation of broiler and layer chicks.

Part 1 Parental influences and embryo development

Part 1 opens with a chapter that reviews genetic selection to improve reproductive traits in chickens. Chapter 1 begins by first focusing on the layer breeding programme structure, then moves on to discuss areas such as egg production, liveability, resilience and adaptability. It also examines how genetic selection can affect egg quality, nesting behaviour and various reproduction traits as well as hatchability, fertility and sperm quality. A section on the molecular tools used is also included, before the chapter concludes with an overview of potential future research trends in the area as well as a section that emphasises the importance of improving reproductive traits to further improve animal health.

The subject of Chapter 2 is managing breeder poultry flocks to optimise hatchability and chick health. The chapter begins by providing an overview of different forms of mating behaviour, such as natural mating behaviour and

mating behaviour in a broiler breeding house. It moves on to review housing systems and the methods that can be used to improve hatchability and chick health. A section on male-to-female ratio is also provided, focusing specifically on common male-to-female ratio practice and adjusted male-to-female ratio. The chapter reviews the use of spiking methods such as spiking young males and intra-spiking. A section on improving broiler breeder nutrition is also provided, followed by an overview of feed restriction and feeding programs. The importance of bodyweight management in female and male broiler breeders is also described, before concluding with an overview of why good feather cover quality is important during the production period.

The next chapter focuses on advances in understanding the development of defences against pathogens in the chicken egg. Chapter 3 starts by providing an overview of the basic components of the egg, followed by a breakdown of the key events that occur during the egg's formation. The chapter provides a summary of how antimicrobial proteins and peptides contribute to the egg's defence, followed by an overview of how each egg component can also contribute to the egg's defence with a special focus on the cuticle through the inclusion of a case study. The chapter also describes the embryo derived structures that are involved in the egg's defence.

Chapter 4 discusses assessing poultry semen quality by examining some of the semen evaluation techniques and fertility determination procedures currently used on breeder farms. The chapter begins by examining the biology of fertilisation, focusing on fertilisation versus fertility and how eggs are formed during fertilisation. It moves on to analyse the importance of semen evaluation and provides an analysis of how semen evaluation techniques can be categorised into subjective and objective categories based on how the data is obtained. The chapter also describes the current techniques used for determining fertility, before concluding with an overview of how on-farm semen evaluation remains crucial in poultry breeding management.

Chapter 5 draws attention to the key stages of embryo development in poultry. The chapter first uses nomenclature to describe the different phases of embryonic development, which is then followed by an overview of fertilisation. The chapter also covers intra-uterine development, focusing on zygotic gene activation and the formation of primordial germ cells and the role of these primordial germ cells in reproduction. A section on embryo development during the incubation period is also provided, followed by a discussion of endocrine control systems and maternal thyroids. The chapter also describes growth and development of the embryo E10-E15, the E16-E19 plateau phase and how extra embryonic fluids can influence compartment development. The chapter also provides an overview of the hatching process, drawing specific attention to synchronous hatching and physiology including the endocrinology of hatching.

The next chapter focuses on chicken egg storage and transport. Chapter 6 first describes how both egg quality and embryo development can influence the effect of egg storage. The chapter also considers practical aspects of using storage temperature, humidity and egg turning to keep eggs in good condition, with some reference to particular aspects of egg hygiene and egg transport. A case study describes the work at Aviagen developing a robust method to deliver short periods of incubation during egg storage to improve the hatchability of stored eggs.

The subject of Chapter 7 is in ovo sexing in poultry chicks. The chapter begins by first highlighting the key issues and challenges of in ovo sexing, then moves on to provide three methods for in ovo sexing through the inclusion of case studies. The case studies section highlights the use of hyperspectral analysis, how endocrinology is crucial for analysing certain components in the allantoic fluid of a chicken embryo, the use of DNA analysis and also mass spectrometry. The chapter also provides an overview of research projects whilst also indicating methods that need to be developed further, such as vibrational spectroscopic methods, magnetic resonance imaging, gene editing, avian egg odour detection and protein quantification.

The final chapter of Part 1 expands on topics previously discussed in Chapter 7 by addressing in ovo vaccination of chicken eggs. Chapter 8 first discusses the history and evolution of in ovo vaccination, then goes on to highlight the various interacting factors of in ovo vaccination success. A section on additional interactions and considerations are also provided, focusing specifically on egg candling for viability, chick handling and welfare, labour efficiencies and quality assurance. The chapter concludes by providing an overview of the challenges that need to be overcome in order to develop more efficient vaccines for in ovo use.

Part 2 Incubation

Part 2 opens with a chapter that focuses on the principles and techniques of incubator design for poultry eggs. Chapter 9 begins by first reviewing the key factors that need to be controlled during the incubation process, individual sections are provided for factors such as temperature, heat production and transfer, positioning and turning of eggs as well as the importance of maintaining moisture, carbon dioxide, oxygen and heat levels through ventilation. The chapter also includes an overview of cooling techniques, then moves on to discuss incubator machine design issues. A section on potential hatcher design issues is also provided, followed by an overview of how the hatching process can be managed and what hatchers should look like for a successful incubation process.

Chapter 10 draws attention to the effects of incubator temperature on embryo and post-hatch chick development. The chapter first compares single

stage incubation to multiple stage incubation, then moves on to highlight embryonic heat requirements. It moves on to review the effect of temperature on hatchling quality and growth, then discusses how thermal manipulation can be used to improve embryo development. The influence of embryonic temperature on organ development is also addressed, drawing specific attention to bone and heart development, endocrine development and the use of thermoregulation. The chapter also discusses how embryonic temperature can affect muscle and adipose tissue development, intestine development and its effect on the immune system. A section on incubation temperature effects on incidence of ascites is also provided, followed by an overview of temperature effects on the integument system.

Chapter 11 focuses on understanding the effects of light on embryo and post-hatch chick development. It begins by assessing the role of monochromatic light on the growth of meat-type birds, focusing specifically on the effect of post-hatch and in ovo monochromatic photostimulation on broilers. The chapter moves on to review the effect of in ovo green light photostimulation on somatotrophic axis activity in embryos and the post-hatch period. The chapter also suggests that embryos are highly sensitive to green light photostimulation, a phenomenon that can be a tool for acceleration of muscle growth in meat type birds. It concludes by highlighting the importance of improving poultry meat production through different external manipulations.

The next chapter examines the effects of humidity/air composition on embryo and post-hatch chick development. Chapter 12 describes the physical function of the eggshell as a respiratory organ for the developing avian embryo, focusing on eggshell porosity, water vapour conductance and incubational egg weight loss and the influence of incubational temperature and airflow. The chapter moves on to review humidity, discussing aspects such as relative humidity and its effects on hatchability, hatchling quality, embryo metabolism and embryonic and post-hatch chick development. It also addresses the influences of breeder age on the effects of relative humidity on embryonic development. A section on vital gases is also provided, specifically focusing on oxygen and carbon dioxide.

Chapter 13 examines the role of egg turning in embryo development. The chapter identifies the four main aspects of turning deemed crucial to successful integration. It first focuses on turning during the egg storage period, then moves on to highlight critical periods during the incubation period. A section on turning frequency during incubation is also included, followed by a discussion on the influence of the turning angle during incubation. A section on signs of turning failure is also provided.

The final chapter of Part 2 reviews poultry embryo development and skeletal growth. Chapter 14 first provides an overview of embryo bone development, drawing specific attention to early development, the growth of long bones,

hormonal regulation and developmental fluctuating asymmetry. The chapter moves on to assess incubation factors that can effect skeletal development, such as temperature, oxygen, carbon dioxide and ventilation, light, egg storage and SPIDES effects on bone and how incubation can effect vertebral development and chondronecrosis with osteomyelitis. Stressful conditions during transportation to the farm is also touched upon.

Part 3 Managing the hatching stage

The final part of the book begins with a chapter that examines alternative hatching systems for broilers. Chapter 15 introduces the current alternative hatching systems in use, then uses this introduction to compare alternative hatching systems to conventional ones. The chapter highlights the environmental factors that can influence both types of hatching systems, such as climate, egg position, light, noise as well as dust and the use of disinfectants. It then moves on to review the management factors that can influence conventional and alternative systems, focusing specifically on chicken handling and transport, the removal of second-grade chickens and time to feed and water access. A section on the effects of alternative hatching systems on health, welfare and poultry production is also provided.

Chapter 16 focuses on managing the poultry hatcher environment. The chapter first looks at the embryonic phase, focusing on development, physiology and metabolism of the chicken embryo and how the hatching position, hatch time and hatch window can effect embryonic mortality. Environmental settings within the hatching phase are also examined, such as temperature settings - both high and low - and relative humidity settings in the hatcher as well. Carbon dioxide concentration settings in the hatcher are also examined. The chapter concludes by providing an overview of why monitoring these settings is crucial in developing and maturing the chicken embryo.

The final chapter of the book discusses the key issues in the transportation of broiler and layer chickens. Chapter 17 focuses first on the transportation of newly hatched chicks, then goes on to look at welfare issues that can affect them during pre-transportation and the transportation process itself. The chapter then reviews how these welfare issues can be assessed which is followed by a discussion on how journey times and periods without feed and water can impact newly hatched chicks. A section on thermal stress in transit is also provided, where the use of thermal comfort zones for chicks is assessed, followed by the use of thermal imaging for surface temperature measurement and the use of thermal micro-environments and ventilation. Stocking density is also examined and exposure of chicks to acceleration and vibration is analysed. The chapter also discusses transport pathologies and provides guidance on good practice for animal transportation as well.

Chapter 1

Genetic selection to improve reproductive traits in chickens

David Cavero and Maurice Raccoursier, H&N International, Germany

- 1 Introduction
- 2 Layer breeding programme structure
- 3 Egg production
- 4 Liveability
- 5 Resilience and adaptability
- 6 Egg quality
- 7 Nesting behaviour
- 8 Reproduction traits
- 9 Hatchability
- 10 Fertility
- 11 Sperm quality
- 12 Molecular tools
- 13 Future trends
- 14 Conclusion
- 15 Where to look for further information
- 16 References

1 Introduction

Global egg production increased from 51.13 million metric tons (Mt) in 2000 to 86.67 Mt in 2020 – an average annual growth of almost 3.5% (FAO, 2022). The number of hens worldwide also increased, albeit at a lower rate, from 4.97 to 7.90 billion hens (2.9% yearly) in the same time frame, which reflects higher productivity on the part of hens.

Disease control, modern housing systems and the use of balanced compound feed have contributed to highly improved performance in the layer industry. However, probably the most significant contribution has come from genetic improvement of birds, which has had a positive and cumulative effect over time. Genetic progress has been documented in experiments designed

especially for broilers and turkeys (Havenstein, 2006), in layers (Jones et al., 2001; Pelletier et al., 2014) and across different farm animal species (Hill, 2008).

The layer breeding industry has substantially contributed to the success of the poultry industry with the development of genetically improved birds. Consistent and continuous improvements in multiple traits such as increased egg production, improved feed efficiency, adaptation of egg quality to consumer preferences and improved robustness and liveability have led to high-performance birds with extended single productive cycles over 100 weeks.

Whilst the focus remains on maximising genetic potential for producing high-quality protein at a competitive cost, additional requirements of the egg industry, changing consumer habits and public opinion related to animal welfare and environmental load are being taken into consideration in order to guarantee the sustainability of egg production. The concept of sustainable development was described by the United Nations Brundtland Commission Report (Brundtland, 1987) as 'meeting the needs of current generations without compromising the ability of future generations to meet their own needs'. As Neeteson et al. (2020) explained, breeding contributes to sustainability by delivering genetic potential to match environmental (through lower resource utilisation and carbon footprint), economic (through increased profitability of the wider industry) and welfare requirements (through meeting societal requirements).

Breeding companies must look beyond current requirements and anticipate future needs and opportunities in the medium and long term. Breeding goals are broad and holistic, oriented towards optimising the use of resources, reducing the production of waste and carbon footprint, increasing food security and food quality, and enhancing the economic and social well-being of producers, consumers and society, as well as the welfare and well-being of the birds.

This chapter reviews these developments in the layer breeding industry, focusing on the current performance of commercial strains as well as selection criteria that might be significant in the future. Special attention is given to reproduction traits and the impact of other important traits on hatchability results. Additionally, the incorporation of new traits, the application of new evaluation methods and future improvements in breeding programmes are discussed.

2 Layer breeding programme structure

Since World War II, poultry production has developed from small scale, local enterprises to an economically important branch of agriculture (Fröhlich et al., 2012). Prior to the introduction of modern breeding, laying hens seldom

Index

- Acrosome 103
- Adrenocorticotropin (ACTH) 53
- AI. *see* Artificial insemination (AI); Artificial intelligence (AI)
- Air cell 376-378
- Air velocity 236
- Albumen 71, 74, 78-79
- Allantoic fluid 180
- Alternative hatching systems, broilers
 - conventional vs. alternative systems, environmental factors 403
 - climate 406-407
 - dust and use of disinfectants 409-410
 - egg position 407
 - light 407-408
 - noise 408-409
 - conventional vs. alternative systems, management factors
 - chicken handling and transport 410-411
 - removal of second-grade chickens 411-412
 - time to feed and water access 412-414
 - current alternative hatching systems 402-403
 - effects of different hatching systems 415
 - effects of health, welfare and production 414
 - behaviour 418-419
 - hatchability and chicken quality 416
 - health and welfare 417-418
 - mortality and performance 417
 - effects of time hatching vs. first feed intake vs. percentage 412
 - hatching systems, based on fast-growing broiler chickens 404-405
 - overview 401-402
 - synopsis 419
 - time after hatching vs. feed intake 413
 - time after start of incubation and heat production 414
- Animal breeding programmes 27
- Animal Welfare Act 174
- Area pellucida 107
- Artificial insemination (AI) 87-89
- Artificial intelligence (AI) 188
- AvBD-11 79
- AvBDs 79
- Aviagen hatcheries 161
- Blastoderm 333
- Bone developmental defects 343
- Breeder poultry flocks, hatchability and chick health
 - body weight, females and males 54-55
 - feather cover 55-56
 - feeding programs 53-54
 - feed restriction 53
 - housing system 38-41
 - floor eggs prevention 41-42
 - male-to-female ratio
 - adjusted ratio 44-45
 - common practice 42-43
 - mating behaviour
 - in broiler breeder house 37-38
 - natural mating behaviour 36-37
 - nutrition
 - crude protein/AA level rearing 47
 - crude protein level females laying phase 48-49
 - crude protein level males rearing 48
 - special male diet 50-53
 - vitamins and trace minerals laying phase 49-50
 - overview of 35-36
 - spiking
 - intra-spiking 46-47
 - young males 45-46
- Broiler breeder management 35
- Cage-free systems 18, 19
- CAMs. *see* Chorioallantoic membranes (CAMs)
- Candling fertility 89
- Carbon dioxide (CO₂)
 - atmospheric concentrations, embryogenesis, hatchability and post-hatch performance 319-320

- changes in incubator concentrations,
 - embryogenesis and hatchability 318-319
- changes in production, developing
 - embryo on incubator concentrations and influences of temperature 317-318
- embryos and hatchlings to elevated atmospheric concentrations and influences of genotype 320-321
- Cargill Animal Nutrition 413
- CASA. *see* Computer-assisted-semen-analysis (CASA)
- CASI. *see* Cell-autonomous sex identity (CASI)
- Cell-autonomous sex identity (CASI) 116
- Chegg system 175
- Chemometric methods 185
- Chicken egg storage
 - blastoderm changes 143-145
 - egg quality changes 145
 - albumen 146-147
 - the cuticle 146
 - mottling 147-148
 - vitelline membrane 147-148
 - egg shell contamination and disinfection 153-154
 - improving embryos survival
 - storage temperature and humidity 148-150
 - overview of 141-143
 - short periods of incubation 154-155
 - during egg storage
 - machines 161-162
 - field trials 161
 - future trends 162
 - replicated experiments 155-161
 - transport 152-153
 - turning eggs 150-152
- Chondrocytes 347-348
- Chorioallantoic membranes (CAMs) 78, 124, 376
- Cloaca strokes 94
- Commercial incubators 237
- Computational fluid dynamics (CFD) 448
- Computer-assisted-semen-analysis (CASA) 92, 93
- Conventional hatching system 409
- Crack detectors 16
- Crossbreds 12, 13
- Day-old chicks 426, 429
- Defences development, pathogens in chicken egg
 - antimicrobial molecules and mechanisms of action 74
- contribution, egg to egg's defence 74-75
 - albumen 78-79
 - the cuticle 75-77
 - eggshell matrix 77-78
 - eggshell membranes 78
 - vitelline membrane 79
 - the yolk 79-80
- egg
 - basic components 70-71
 - formation 71-74
 - embryo-derived structures 80
 - overview of 69-70
- Detrimental effect of, abnormal temperatures 256
- Diploid zygote 105
- Disease control 199-200
- Disinfectants 409-410
- DNA 26
- Dual-purpose chickens 190
- Dynamic stiffness 16
- Dynamic stiffness measurement (Kdyn) 16, 17
- Early bone development
 - bone-encoding genes 345-346
 - bony layer 346
 - cellular regulation 345
 - embryo day (ED) 346
 - embryogenesis 345
 - osteoblast cells vs. capillaries 346-347
 - osteogenic 346
- Ectoderm 112
- EEBC. *see* Extra-embryonic body cavity (EEBC)
- EEC. *see* Endodermal epithelial cells (EEC)
- Effects of humidity/air composition, embryo and post-hatch chick development
 - humidity 308-313
 - overview 303
 - physical function of eggshell, a respiratory organ for avian embryo 304-308
 - synopsis 321-322
 - vital gases
 - carbon dioxide 317-321
 - oxygen 313-317
- Effects of incubator temperature, embryo and post-hatch development
 - body, yolk and yolk-free body mass 261
 - embryonic heat requirements
 - blood flow 258

- broiler line 258-259
- genetics company 258
- late embryonic vs. incubator air temperature 257
- overheating 258
- oxygen concentration 258
- thermal conductivity 257-258
- embryo shell temperature 259
- hatchling quality and growth
 - broiler performance 259-260
 - optimal embryo temperature 261
 - overheating effect 260
- incubational thermal manipulation 261-262
- incubation temperature effects on incidence of ascites 268-269
- influence of organ development 262
 - bone 263
 - endocrine 263-264
 - heart 263
 - immune system 267-268
 - intestine 267
 - muscle and adipose tissue 265-267
 - thermoregulation 264-265
- overview 255-256
- single-stage vs. multiple-stage incubation 256-257
- synopsis 270
- temperature effects on integument system 269
- Effects of light, embryo and post-hatch chick development
 - body weight of male broiler, different light spectra 281
 - monochromatic light, growth of meat type birds
 - in ovo monochromatic photostimulation, growth and development 281-288
 - post-hatch monochromatic photostimulation, growth and development 280-281
- overview 279-280
- in ovo GL photostimulation affects, core temperature of incubated eggs 283
- in ovo GL photostimulation on somatotrophic axis activity 288-297
- percentage BW of egg weight and pectoralis muscle weight 284
- synopsis 297-298
- Egg fertility 35, 47, 50
- Eggshell membrane fibres 71
- Eggshell temperature (EST) 344, 382-383
 - air velocity 406
 - climatic factor 407
 - on-farm hatching 406
 - relative humidity (RH) 406-407
 - X-Treck system 406
- EggXYt method 188
- EGK-X. see Eyal-Giladi stage X (EGK-X)
- Electrical current flow 191
- Embryo development stages, poultry
 - chicken embryo, chronology 134-140
 - E16-E19 plateau phase 118-119
 - embryo E10-E15, growth and development 117-118
- endocrine control systems 116-117
- extra-embryonic fluids and compartment
 - amnion and chorion formation 122-123
 - amnion functions 123
 - chorion and allantois membranes 123-124
 - sub-embryonic fluid formation 119-120
 - yolk sac and extra-embryonic coelom 120-122
- hatching
 - endocrinology of 125-126
 - synchronous hatching 124-125
- during incubation
 - embryo and extra-embryonic tissue development 109-110
 - first day of incubation 110-111
 - germ layers development 111-113
 - morphological differentiation, embryo E1-E9 113-116
- intra-uterine development
 - Eyal-Giladi and Kochav stage V-XII 108-109
 - primordial germ cells 106-108
 - zygotic gene activation and formation 105-106
- maternal thyroids 116-117
- nomenclature 102-103
- one-cell embryo/zygote formation 103-105
- overview of 101-102
- Embryonic blood vessel system 186
- Embryonic mortality 376-377
- Embryos 235
- Embryo temperature 382
- Endoderm 112
- Endodermal epithelial cells (EEC) 112

- External pipping (EP) 376-377
- Extra-embryonic body cavity (EEBC) 111, 112, 114, 120
- Eyal-Giladi stage X (EGK-X) 105-106, 108
- Factors controlling incubation 255
- Failure to turn eggs 338
- FCR. *see* Feed conversion ratio (FCR)
- Fecundity rate 25
- Feed conversion ratio (FCR) 13, 447
- First-grade chickens 410
- Floor house system 39
- Fluorescence spectroscopy 184-187
- Food and Agriculture Organization (FAO) 425
- Footpad dermatitis (FPD) 417-418
- Forced-ventilated hatchers 409-410
- Formaldehyde 154
- Fourier transform infrared (FT-IR) spectroscopy 183-184
- FPD. *see* Footpad dermatitis (FPD)
- FT-IR. *see* Fourier transform infrared (FT-IR) spectroscopy
- Gastrulation 111
- GD region. *see* Germinal disc (GD) region
- Genetic correlation 12, 17, 25
- Genetic selection, chicken reproductive traits
- adaptability 12-13
 - egg production 7-11
 - egg quality 13-18, 21
 - fertility 22-24
 - future trends 26-27
 - hatchability 19-22
 - layer breeding programme structure 4-7
 - molecular tools 26
 - nesting behaviour 18-19
 - overview of 3-4
 - reproduction traits 19-20
 - resilience 12-13
 - sperm quality 24-25
- Genetic switch 181
- Genome-wide association studies (GWAS) 26
- German Animal Protection Act 187
- Germinal disc (GD) region 90, 91, 95
- Germ plasm 107
- Glycogen 122
- Good-quality chicken requirements 233-234
- Gram-negative bacteria 79
- Growth of long bones
- chondrocytes 347-348
 - ED 14 to ED 19 348
 - epiphyseal chondrocytes 347
 - hypertrophic zone chondrocytes 348
 - hypoxia 348-349
 - transforming growth factor (TGF-) 347
- GWAS. *see* Genome-wide association studies (GWAS)
- Hanging droplet method 93
- HatchCare 402
- Hatching chicks 331
- Hatch time and hatch window
- affected factors 380
 - background noise 380-381
 - chicken embryos 378
 - communication 380
 - monitoring batches of eggs 379
 - phases 379
 - physiological and developmental traits 379
- Hatch window 125, 409
- Haugh unit 17
- Heat production 258-259
- Hemizygote sex chromosomes (ZW) 178, 181
- Hensen's node 111
- Heritability 6, 8, 10, 23
- Higher late embryonic mortality 387
- High temperatures
- artificial incubation 383
 - body temperature 386-387
 - control EST 384, 386
 - effects of EST treatment 385-386
 - embryonic O₂ availability 386
 - high EST 384-387
 - metabolic rate and O₂ requirement 383-384
 - O₂ availability 386
 - relative organ weights and functioning 384-385
- Homozygote sex chromosomes (ZZ) 178, 181
- Humidity
- age, effects of relative humidity on embryonic development 312-313
 - egg water content, production and loss during embryonic development 308-309
 - egg water loss requirement 310
 - relative humidity 309
 - embryo metabolism and embryonic and post-hatch chick development 311-312

- hatchability and hatchling quality 310-311
- Hypereye method 181
- Hypothalamus-hypophyseal complex 117
- Hypoxic conditions 376
- IBV vaccination 53
- Incubation 233
- Incubation on post-hatch performance 388
- Incubation time 21
- Incubator design for poultry eggs, principles and techniques
 - cooling techniques 242-244
 - effect of air velocity, machine temperature 236
 - hatcher 251-252
 - hatcher design issues 246-247
 - incubator machine design issues 244-246
 - managing the hatching process 247-251
 - overview 233-234
 - positioning and turning of eggs 237-238
 - temperature, heat production and heat transfer 234-237
 - ventilation 238-240
 - CO₂ and O₂ 240-241
 - managing heat 241-242
- Incubator temperature 382
- Influence heat transfer 235-236
- Inner perivitelline layer (IPL) 90, 96, 103
- In ovo monochromatic photostimulation, growth and development
 - commercial hatcheries 281-282
 - GL photostimulation 282
 - monochromatic GL 282
 - in ovo GL photostimulation 286-287
 - ovo-treated groups 282-283
 - proliferation and differentiation of myoblasts 285
 - satellite cells 284-285
 - skeletal muscle fibers 284
- In ovo sexing, poultry chicks
 - DNA analysis 178-180
 - endocrinology 176-178
 - future research 181-183
 - avian egg odour 188-189
 - gene editing 188
 - magnetic resonance imaging 188
 - protein quantification 189
 - vibrational spectroscopic methods 183-187
 - future trends 190-192
 - hyperspectral analysis 174-176
 - key issues and challenges 170-174
 - mass spectrometry 180
 - overview of 169-170
- In ovo vaccination, chicken eggs
 - additional interactions and considerations for in ovo vaccination
 - chick handling and welfare 223-224
 - egg candling for viability 223
 - labor efficiencies 224-225
 - quality assurance 225-226
 - future trends 226-227
 - history and evolution of 198-200
 - interacting factors (see Interacting factors, in ovo vaccination success)
 - overview 197-198
 - USDA licensed manufacturer, December 2021 198
- Interacting factors, in ovo vaccination success
 - asepsis of vaccine mixing and handling 218-219
 - automation 222-223
 - device adaptation, commercial egg incubation
 - eggs for human consumption 200-201
 - incubator egg tray design 202
 - normal production 203
 - production scope of, commercial hatchery 201-202
 - transferred baskets 202
 - device and eggshell interface 203-204
 - egg handling during injection/transfer 219-222
 - embryo orientation and late-stage embryo development 204-206
 - hatchery hygiene and biosecurity 211-213
 - immunity function of vaccine specifications 216-218
 - importance of compartmental delivery 213-216
 - sanitation for 210-211
 - shell penetration and injection needle 206-208
 - vaccine specifications and delivery 208-210
- Internal pipping (IP) 376-377
- IPL. see Inner perivitelline layer (IPL)
- Japanese method 170

- Laser-induced fluorescence (LIF) 187
 LED. *see* Light-emitting diode (LED)
 Leg and locomotion problems 343
 LIF. *see* Laser-induced fluorescence (LIF)
 Light-emitting diode (LED) 280
 Light quality 279
 Light spectra affect growth 280
 Lysozyme 79
- Magnetic resonance imaging (MRI) 188
 Magnum 71
 Malposition 377-378
 Managing the poultry hatcher environment
 - embryonic phase
 - development, physiology and metabolism 376-377
 - hatching position 377-378
 - hatch time and hatch window 378-381
 - environmental settings, hatching phase 381
 - CO₂ concentration settings in the hatcher 389-391
 - high temperatures 383-387
 - low temperatures 387-388
 - relative humidity settings 388-389
 - temperature settings 381-383
 - overview 375-376
 - synopsis 391
- Marek's disease 199
 Master-endocrine-regulator 117
 Meat-type birds 279
 Meiotic cell 104, 105
 Mesoderm 113
 Mesoderm cells cluster 111
 Modern commercial incubators 235
 Molecular genetic methods 181
 Monitoring batches of eggs 379
 MRI. *see* Magnetic resonance imaging (MRI)
- Near-infrared (NIR) range 174
 NestBorn 403
 NIR. *see* Near-infrared (NIR) range
 Non-linear moisture loss 240
 Nonviable embryos 89
- OCT. *see* Optical coherence tomography (OCT)
 One2Born 403
 OPL. *see* Outer perivitelline layer (OPL)
 Optical coherence tomography (OCT) 184
 OPVL. *see* Outer perivitelline membrane (OPVL)
- ORBEM 188
 Outer perivitelline layer (OPL) 91, 95, 96
 Outer perivitelline membrane (OPVL) 103
 Over-mating 38
 Ovocalyxin-32 (OCX-32) 75, 78
 Ovocalyxin-36 (OCX-36) 75, 78
 Ovoinhibitor 79
 Ovomucin 79
 Ovomuroid 79
 Oxygen (O₂)
 - effects of atmospheric concentrations, embryo and hatchling 316-317
 - eggshell porosity, air cell tension and transition from chorioallantois vasculature to pulmonary respiration 313-314
 - eggshell porosity, embryo metabolism and temperature on embryonic consumption and hatchling quality 314
 - embryo and hatchling to environmental concentrations 315-316
- Packed cell volume procedure (PCV) 93
 Palisade layer 72
 Parathyroid hormone-related protein (PTHrP) 348
 Patio 402-403
 PCR method. *see* Polymerase chain reaction (PCR) method
 PCV. *see* Packed cell volume procedure (PCV)
 PGCs. *see* Primordial germ cells (PGCs)
 Phenotypic plasticity 344
 Physical function of eggshell, respiratory organ for developing avian embryo
 - eggshell porosity 304-305
 - influences of incubational temperature and airflow 306-307
 - measurement of internal egg temperature 307-308
 - water vapor conductance and incubational egg weight loss 305-306
- PLANTegg process 178, 180
 Polymerase chain reaction (PCR) method 178, 180
 Poultry embryo development and skeletal growth
 - effects of temperature 344
 - eggshell temperature (EST) 344
 - embryo bone development

- developmental fluctuating asymmetry 349-350
- early development 345-347
- growth of long bones 347-349
- hormonal regulation 349
- environmental factors 344-345
- impact of modifications 345
- incubation factors, affect skeletal development 350-351
- egg storage and SPIDES effects on bone 359-360
- light 358-359
- oxygen, carbon dioxide and ventilation 355-358
- stressful conditions, transportation to farm 361-362
- temperature 351-355
- vertebral and chondronecrosis, osteomyelitis 360-361
- leg and locomotion problems 343
- ossification issues 343
- phenotypic plasticity 344
- synopsis 362-363
- Poultry semen quality
 - fertility determination 95-96
 - fertilization
 - egg formation and vs. fertility 90-91
 - overview of 87-89
 - semen evaluation
 - categories 92-93
 - encompassing perspective 94-95
 - purpose 92
- Primordial germ cells (PGCs) 105-107
- Proof of concept 173
- Purebreds 12, 13

- QTC. *see* Quality time concept (QTC)
- Quality of day-old chickens 401
- Quality time concept (QTC) 40

- Raman effect 184
- Raman spectroscopy 174, 184-187
- Red Jungle Fowl 36
- Relative humidity (RH) 149, 150
- Reproductive fitness 20
- Resonance effect 184
- RH. *see* Relative humidity (RH)
- Role of egg turning in embryo development
 - advantage 96 vs. 24 turns daily 336
 - critical periods during incubation 333-335
 - effect of frequency 337
 - effect of turning angle and daily turning frequency 338
 - effect of turning incubation on percentage 332
 - effect on absence of turning 334
 - overview 331-332
 - signs of turning failure 338
 - synopsis 338-339
 - turning angle during incubation 337-338
 - turning during storage 332-333
 - turning frequency during incubation 335-337
- Role of light, biological activity 279

- Salmonella enterica* 70
- Second-grade chickens 411
- SED. *see* Small end down (SED)
- SEF. *see* Sub-embryonic fluid (SEF)
- Seleggt process 178, 179
- Selenium (Se) 50, 52
- Semen quality traits 88
- SEU. *see* Small end up (SEU)
- Shell strength 16
- Shell thickness 16
- Short periods of incubation during egg storage (SPIDES) 21, 156-162
- Single nucleotide polymorphisms (SNPs) 5, 6, 26
- Sinus terminalis 114
- Skip-a-day program 54
- Small end down (SED) 150
- Small end up (SEU) 150, 152
- SmartStart 402
- SMI. *see* Sperm motility index (SMI)
- SNPs. *see* Single nucleotide polymorphisms (SNPs)
- Solid-phase microextraction/gas chromatograph-mass spectrometry (SPME/GC-MS) 189
- Somatopleure membrane 112
- SOPs. *see* Standard operating procedures (SOPs)
- Spectroscopic measurement 183
- Sperm concentration 25
- Sperm motility 25, 92
- Sperm motility index (SMI) 92
- Sperm quality analyzer (SQA) 92, 93
- Sperm storage tubules (SSTs) 91, 94, 96
- SPIDES. *see* Short periods of incubation during egg storage (SPIDES)

- Splanchnopleure membrane 112
- SPME/GC-MS. *see* Solid-phase
 microextraction/gas chromatograph-
 mass spectrometry (SPME/GC-MS)
- SQA. *see* Sperm quality analyzer (SQA)
- SSTs. *see* Sperm storage tubules (SSTs)
- Standard operating procedures (SOPs) 92,
 93
- Sub-embryonic fluid (SEF) 114, 119-120
- Support vector machines (SVM) 185
- Sustained fertility 91
- SVM. *see* Support vector machines (SVM)
- Tandem mass tags (TMT) 189
- Technology readiness level 171, 172
- Temperature 255-256
 bone development 351
 commercial conditions 353
 early incubation 351-352
 exposing embryos to high
 temperatures 352-353
 hatching time and hatch
 window 354-355
 modifications of incubation 352
 O₂ consumption 353
 reports indicate 353
 single and multi-stage
 incubation 353-354
 skeletal and bone development 354
- TeraEgg technology 189
- TH. *see* Thyroid hormones (TH)
- Thermal comfort zones
 biological requirements 443
 deep body temperature 447
 environmental temperatures 441
 heterogeneity 445
 Het Spelderholt 441
 HSUS report 442
 metabolic heat productions 443-444
 mortality 444-445
 physiological variables 446
 produced and holding periods 446
 rectal temperatures of newly hatched
 chicks 442
 thermal gradients 445
 thermal stress 442
 thermoregulation 444-446
 transportation environments 443
 ventilation 442
- Thermal manipulation 266-267
- Thyroid hormones (TH) 117
- Time-resolved laser-induced fluorescence
 spectroscopy 187
- Tinytag data loggers 161
- TMT. *see* Tandem mass tags (TMT)
- Traditional system 238
- Transportation issues of, broiler and layer
 chicks
 assessing welfare of newly hatched
 chicks 434
 exposure of chicks to acceleration and
 vibration 451-452
 guidance on good practice
 animal welfare science and policy 455
 commercial guidelines, handbooks,
 manuals and advisory
 publications 454
 Farm Animal Welfare Committee
 (FAWC) 454-455
 poultry-specific guide 454
 Regulation EC 1/2005 453-454
 sources 454
 journey times and periods without feed
 and water 434-440
 effects of parental age 436-437
 European legislation (EC 1/2005)
 prohibits journeys 435
 genetic predecessors 437
 investigated effects 436
 long-distance transport 436-437
 metabolic rate 438, 439
 novel husbandry systems 440
 physiological challenges 434
 physiology 438-439
 pre-placement feed (PPF) 439
 stress 436
 thermal conditions 434
 use of metabolic reserves 435
 newly hatched chick boxes/trays/baskets/
 crates, commercial use 427
 overview 425-426
 stocking density
 chick box design 450
 deep body temperature 451
 space allowance 449-450
 transport legislation 449
 synopsis 455-457
 thermal stress in transit 440-441
 surface temperature
 measurement 447
 thermal comfort zones 441-447
 thermal micro-environments and
 ventilation 448-449
 transportation of newly hatched
 chicks 426-428
 transport pathologies 452-453

- welfare issues affecting newly hatched chicks, pre-transport
- behavioural and production consequences 431-432
 - commercial setting 429
 - investigate impact processes of commercialization 429-431
 - post-hatch treatment 429
 - production systems 428
 - time window 429
- welfare issues relating to transport of newly hatched chicks
- Humane Society of the United States (HSUS) 432-433
 - research recommendations 433
 - Ross Broiler Handbook 433
 - stress 432
 - thermal conditions 432
- True fertility 89
- Turned eggs angles 337
- Turning treatments 331-332, 336
- UK, chick transportation 426
- Ultraviolet (UV) light 154
- Utero-vagina junction (UVJ) 91, 96
- Uterus 105
- UVJ. *see* Utero-vagina junction (UVJ)
- Vaccination method 197
- Values of maximal heat production 258
- Ventilation
- managing heat 241-242
 - moisture loss 239
 - not spraying 239-240
 - properties and demands of embryo 238
 - relative humidity (RH) 240-242
 - removal of CO₂ and supply of O₂ 240-241
 - spraying 239-240
- Vertical crystal layer 72
- Viable embryos 89
- Vital gases
- carbon dioxide (CO₂)
 - atmospheric concentrations,
 - incubation on embryogenesis,
 - hatchability and post-hatch performance 319-320
 - developing embryo on incubator concentrations and influences of temperature 317-318
 - embryos and hatchlings to atmospheric concentrations and influences of genotype 320-321
 - incubator concentrations, embryogenesis and hatchability 318-319
 - oxygen (O₂)
 - altitude effects of atmospheric concentrations, embryo and hatchling 316-317
 - eggshell porosity, air cell tension and chorioallantois vasculature to pulmonary respiration 313-314
 - eggshell porosity, embryo metabolism and temperature on embryonic consumption and hatchling quality 314
 - embryo and hatchling to environmental concentrations 315-316
- Vitamin E 49, 52
- Vitelline membrane 70
- Water evaporation 236
- White Leghorn 16, 19
- World's Poultry Science Association (WPSA) 28
- WPSA. *see* World's Poultry Science Association (WPSA)
- X-Treck 403
- Yolk-free body mass (YFBM) 416
- Yolk sac membrane (YSM) 122, 124
- ZGA. *see* Zygotic Gene Activation (ZGA)
- Zinc 50
- ZP1 103
- ZP3 103
- Zygotic Gene Activation (ZGA) 105