

# Index

Note: page numbers in *italics* refer to figures and tables

- acid dissociation constant ( $K_a$ )  
 32
- air quality  
 filtration systems 270  
 incubators 262  
   control 270–2  
 IVF laboratory 241  
 particle counts 270–1  
 volatile organic compounds  
 271–2
- alanine 11
- alanyl-L-glutamine 10–11, 52  
 ammonium release in culture  
 media 105
- albumin  
 disease transmission risk  
 71–2  
 functions 71  
 use in embryo culture media  
 71–2  
 zona pellucida penetration 75
- albuminar-5 71–2
- aliphatic amines, zwitterionic  
 30, 32
- alpha globulin 72–4, 73  
 addition to human serum  
 albumin 73–4  
 blastocyst formation 73–4  
 zona pellucida penetration 75
- altitude  
 carbon dioxide levels 267  
 effects on pH 146–8  
 incubators 267  
 oxygen levels 267
- amino acids 95–104  
 addition to media 6  
 altered 30  
 ammonia release 10–12,  
 104–6  
 antioxidant role 101  
 cell signaling regulation  
 101–2  
 chelator role 101  
 concentration in media 10  
 culture media for oocyte  
 maturation 159  
 deamination 10–11
- embryo culture media 98–9,  
 106
- embryo requirements 100
- embryonic stem cell cultures  
 102–3
- epigenetic regulation 103–4  
 functions 95  
   traditional 99–101
- intracellular pH regulation 100
- media content 9–12, 51–2  
 negative effects 10–11
- osmolytes 57, 99–100  
   in culture media for oocyte  
 maturation 159
- pluripotency 102–3
- protein substrate 96, 101
- in reproductive tract 95–7, 97
- sensors 101–2
- transport  
 mechanisms 52  
   in oocytes/embryos 97–8
- transporters 101–2
- see also* essential amino acids  
 (EAA); non-essential  
 amino acids (NEAA)
- ammonia/ammonium ions  
 in embryo culture media  
 105–6
- embryos 105  
 in media 52–3  
 release from amino acids  
 10–12, 104–6
- antibiotics in media  
 classification 186–7  
 content 14
- anti-mullerian hormone  
 (AMH) 157
- antioxidants  
 amino acids role 101  
 culture media for oocyte  
 maturation 159–60  
 media content 13, 53
- armadillo studies  
 monozygotic twinning 84–5
- placentation 89
- polyembryony 85
- quadruplets 84
- assisted reproductive  
 technologies (ART)  
 affordable 246–7  
 center distribution in  
 developing countries 100  
 commercial issues 278  
 complications following  
 oocyte retrieval 252  
 developing countries 245  
 history of regulation 183  
 ovarian hyperstimulation  
 syndrome 252  
 pregnancy complications 253  
 risk minimizing 252–3  
 training of professionals 253
- ATP:ADP ratio in embryos 7
- B2 medium 48
- “back to nature” approach 2, 4  
 development 22–3
- bacterial growth risk in  
 incubators 272
- BES buffer 37
- best practice 62
- beta globulin 72–4, 73  
 addition to human serum  
 albumin 73–4
- blastocyst formation 73–4  
 zona pellucida penetration  
 75
- bicarbonate ions  
 buffering 54, 240  
 Henderson–Hasselbalch  
 equation 54  
 media content 7  
 pH 143–4
- biological antifreeze proteins  
 174
- blastocyst(s)  
 ability to implant 236  
 cryobanking 91  
 culture condition  
 optimization 91  
 elastic properties 198  
 GM-CSF in culture media  
 118–19, 123  
 monozygotic twinning 85, 86

- blastocyst(s) (cont.)  
 culture condition  
 optimization 91  
 splitting 85, 86  
 blastocyst formation 7  
 alpha globulin 73–4  
 amino acids in 95  
 beta globulin 73–4  
 EDTA media content 13  
 metabolism 9  
 zygote development into in  
 oviduct 21  
 blastocyst transfer  
 after co-culture 90  
 monozygotic twinning 82,  
 84, 90  
 co-culture 90  
 timing 88–9  
 blastomeres, size/fragmentation  
 221–2  
 Bovine Oviduct Medium for  
 Embryo Culture  
 (BOME) 23, 24  
 bovine serum albumin (BSA)  
 55  
 fatty acid-free 160  
 bovine zygotes 23–5  
 Braille displays 203–4, 204  
 buffering capacity (pK<sub>a</sub> value)  
 31, 32  
 buffers 30–42, 54–5  
 BES buffer 37  
 bicarbonate ions 54, 240  
 cell development impact 39  
 combination system 34, 38, 41  
 concentration 42, 42  
 cryopreservation 38–41  
 temperature-stable 41  
 DIPSO buffer 34, 38  
 embryos 37–8  
 incubator media 42  
 MES buffer 37  
 oocytes 32–5  
 pH  
 change in media 40  
 control of embryo culture  
 media 143, 152–3, 258  
 phosphate buffered solutions  
 33, 35  
 PIPES buffer 34, 36  
 sperm 35–7  
 TAPSO buffer 34–5  
 TES buffer 34, 36, 38, 41  
 TEST buffer 34–6  
 tricine 37  
 Tris buffer 34–5  
 TYB buffer 36  
 undesired actions 32  
 zwitterionic 35, 55  
 zwitterionic aliphatic amines  
 30, 32  
*see also* HEPES buffer;  
 MOPS buffer  
 calcium ions, media content 7  
 carbon dioxide  
 altitude effects 267  
 gas phase 15  
 incubator levels 261  
 concentration in chamber  
 15, 147, 148, 273  
 control for human embryo  
 culture 148–9  
 monitoring 148–9  
 levels in media 61  
 pH regulation 143–4  
 altitude effects 146–8  
 carbon dioxide sensors 266–7  
 CE certification process in  
 Europe 187–9  
 ancillary human blood  
 derivative quality/safety  
 190  
 Medicinal Dossier 191–2  
 ancillary medicinal  
 substances 187–8  
 Medicinal Dossier for  
 Quality and Safety 191  
 Common Technical  
 Document 191–2  
 Competent Authority  
 process 192–3  
 role 190  
 timelines 192–3  
 Design Dossier 191  
 documentation requirements  
 190–3  
 European Medicines Agency  
 190  
 submission process/  
 timelines 192–3  
 Full Quality Assurance route  
 188–9  
 marketing authorization 190  
 Medical Device Directive  
 189–90  
 Medicinal Dossier for  
 Quality and Safety  
 ancillary antibiotic 191  
 ancillary human blood  
 derivative 191–2  
 Notified Body 187–9  
 design of device 189–90  
 documentation  
 requirements 190–3  
 process 192–3  
 responsibility 188–9  
 timelines 192–3  
 process 192–3  
 Quality Management System  
 189  
 routes to conformity 188  
 timelines 192–3  
 cell cycle duration 225–7  
 cells  
 growth factors/cytokines in  
 communication  
 113–14  
 mechanosensitive 198–9  
 semi-permeable membrane  
 132–3  
 shear stress effects 198–9  
 volume regulation model 137  
 cervix, Toll-like receptor  
 expression by epithelial  
 cells 116–17  
 chelators, media content 13  
 chemokines 115  
 cleavage  
 duration 225–7  
 early 218–21, 224  
 implantation rate 219  
 reverse 223  
 timing 224, 226–7  
 clomiphene citrate 248–9, 249  
 co-culture, blastocyst transfer  
 90  
 contact supplies, media 15  
 Creutzfeldt–Jakob disease  
 (CJD)/new variant  
 Creutzfeldt–Jakob  
 disease (nvCJD) 72  
 cryopreservation 172  
 cooling rates 175  
 equilibrium procedures  
 172–3, 176  
 glass state 173, 180  
 ice-free 173, 180  
 non-equilibrium methods 173  
 rapid cooling 173  
 slow freezing 172–3, 176  
 types 172–3  
 vitrification 173, 180  
 cryopreservation media 172–80  
 buffers 38–41  
 temperature-stable 41  
 carrier solutions 173  
 components 173–4

- cryoprotection 172–4  
*see also* cryoprotectant agents)
- embryo culture media 38–41  
   albumin use 71–2  
   human serum albumin addition 74  
   Serum Substitute Solution 74  
 equilibrium procedures 176  
 HEPES buffer 39–40  
 ice blockers 174  
 mechanism of action 175–80  
 MOPS buffer 39–40  
 phosphate buffered solutions 39–40, 54  
 slow freezing 176  
 TES buffer 41  
 vitrification solutions 178, 179
- cryoprotectant agents 172–4  
   addition to carrier solutions 173  
   combination 175  
   cooling rates 175  
   cytotoxicity 180  
   extracellular 174–5, 179–80  
   intermolecular actions 180  
   intracellular 173–5  
   mechanism of action 175–80  
   non-permeating 174–5, 179–80  
   permeating 173–5  
   physics 174–5  
   properties 175  
   vitrification 180
- culture dishes  
   heating 260–1  
   temperature control 236–7
- cumulus–oocyte complex (COC), human 161
- cyclic adenosine monophosphate (cAMP) 162
- cytokines  
   cell communication 113–14  
   classification 113–14  
   control mechanisms 114  
   embryo development 112–25  
     later fetal/postnatal growth effects 120  
     regulation 118–19  
     sensing/adaptation regulation 121  
   embryo developmental competence 119–22
- embryo programming 119–20
- endometrial synthesis 115–18  
   epithelial cell 115–17  
   leukocytes 117–18  
   steroid-hormone regulation 115–16
- expression 114–15  
   female reproductive tract 116
- functions 114
- maternal tract  
   communication with embryonic 118, 121  
   quality control 121–2
- oviductal production  
   epithelial cell 115–17  
   leukocytes 117–18
- receptor binding 114  
*see also* granulocyte–macrophage colony-stimulating factor (GM-CSF)
- cytoskeleton,  
   mechanotransducers in 199
- CZB culture medium 133–4
- density 57–8  
 density gradient materials 58  
 developing countries 245–6  
 dextrans, zona pellucida penetration 75  
 diapause, embryonic 84–5  
 dichorionic placentation 84, 89  
 DIPSO buffer 34, 38  
 dizygotic triplets 83, 90  
 dizygous twins 80  
 DNA methylation 103, 103–4
- EDTA, media content 13
- electromagnetic fields,  
   incubators 273–4
- Embden–Meyerhof pathway,  
   glucose metabolism 158
- embryo(s)  
   amino acids  
     in development 95–7, 99  
     requirements 100  
     transport 97–8  
   ammonium effects 105  
   ATP:ADP ratio 7  
   autocrine/paracrine factor production 24  
   autonomy 24–6
- bovine and ammonium effects 105
- cell cycle duration 225–7
- cell numbers  
   even 224  
   uneven 225
- chromosomal defects 218
- cleavage  
   duration 225–7  
   early 218–21, 219, 224  
   reverse 223  
   times 224  
   timing 224, 226–7
- cytokines  
   adaptation regulation 121  
   in development 112–25  
   in development regulation 118–19  
   developmental competence 119–22  
   human embryo development regulation 118–19  
   later fetal/postnatal growth effects 120  
   in programming 119–20  
   sensing regulation 121  
   synthesis 118
- de-selection parameters 227
- development  
   amino acids role 95–7, 99  
   cytokine regulation 118–19  
   cytokine regulation of sensing/adaptation 121  
   cytokines and developmental competence 119–22  
   cytokines in 112–25  
   cytokines in human embryos 118–19  
   glutamine in 99  
   GM-CSF in protection from cell stress/apoptosis 123–4  
   GM-CSF role 122–4  
   growth factors in 112–14  
   key events 224  
   methionine in epigenetic regulation 103, 103–4  
   plasticity 121  
   progression 220  
   taurine 99  
   diapause 84–5  
   division cycles 225

- embryo(s) (cont.)  
 divisional synchrony 224, 225  
 donor oocyte-generated 87  
 early cleavage 218–21, 224  
 implantation rate 219  
 elastic properties 198  
 electromagnetic field effects 273–4  
 evaluation algorithm 227  
 extended culture 91  
 female tract communication  
 molecular mechanisms 121  
 fragment appearance/  
 disappearance 221  
 frozen-thawed 88  
 genetic imprinting 120  
 glucose metabolism 56  
 glucose uptake 8  
 human  
 ammonium effects 105  
 cytokines in development  
 regulation 118–19  
 GM-CSF in embryo  
 culture media 122–3  
 incubators for culture  
 148–9  
 osmolality of culture  
 medium 138–9  
 manipulation 240  
 metabolism 8, 56–7  
 microenvironment  
 modulation by  
 macromolecules 77  
 MOPS buffer 38, 55  
 morphokinetic analysis  
 211–30, 226  
 classification criteria 228  
 factors affecting 229  
 multinucleated 222–3, 223  
 non-invasive methods 62  
 nutrient requirements 4  
 observation minimization  
 240–1, 241  
 observation systems in  
 incubators 265  
 oocyte origin 22  
 oxidative damage 53  
 pH of medium 59, 238  
 development success 239  
 stability 237–9  
 platform tilting 200–1, 202  
 preimplantation  
 metabolomic assessment  
 206  
 morphokinetic analysis  
 211–30  
 selection 205–6, 211–30  
 shear stress sensing/  
 reaction to 199  
 preimplantation  
 development  
 amino acids consumption/  
 excretion 11  
 ammonium effects 105  
 apoptosis 123–4  
 cytokines 119–20  
 epigenetic regulation 103  
 glutamine in 99  
 GM-CSF for optimal fetal  
 123  
 time-lapse imaging 211–30  
 programmed cell death 199  
 pronuclear formation/fading  
 220, 226–7, 228  
 safety 26  
 selection 205–6, 211–30  
 blastomere size/  
 fragmentation 221–2  
 dark-field imaging unit  
 with software-based  
 embryo evaluation 215  
 de-selection parameters  
 227  
 Hoffmann modulation  
 contrast imaging unit  
 214  
 image acquisition systems  
 in incubators 215  
 integrated TLI unit 215–16  
 morphokinetic analysis  
 211–30, 226  
 morphological criteria  
 218–23  
 multinucleation 222–3  
 procedure 212–13  
 shear stress sensitivity 240  
 slow with IVF 86  
 stimulation through  
 vibration 201–3  
 temperature sensitivity  
 236–7  
 TES buffer 38  
 time-lapse imaging 211–30  
 annotation parameters 223  
 approaches 213–15  
 clinical benefits 229  
 controlled incubation  
 environment 213  
 differences between  
 available systems 216  
 division cycles 225  
 flexible with modified  
 standard incubator 215  
 integrated unit 215–16  
 light exposure 216, 217  
 monitoring system  
 certified for clinical use  
 215  
 multinucleation 222–3  
 stages of human  
 development 212  
 strategy adaptation 228–9  
 videography in incubation  
 chamber 15  
 time-lapse monitoring 213,  
 215–18  
 annotation parameters 223  
 blastomere size/  
 fragmentation 222  
 early cleavage 219–21  
 light dose 216  
 novel selection/de-  
 selection parameters  
 223–9  
*versus* standard assessment  
 218–23  
 transfer timing  
 before/after genome  
 activation 86–7  
 rate reduction on  
 monozygotic twinning  
 91  
 uterine transfer 85  
 variation and birthweight of  
 offspring 26  
 vibration of cell culture  
 system 201–3  
 in vitro propagation 68  
 in vivo development 68–9  
 embryo culture media  
 albumin use 71–2  
 alpha globulins 72–4  
 amino acids 98–9, 106  
 ammonium levels 105–6  
 beta globulins 72–4  
 bovine zygote cleavage 24  
 buffers 37–8  
 carbon dioxide-enriched  
 environment 143  
 cryopreservation 38–41  
 albumin use 71–2  
 human serum albumin  
 addition 74  
 Serum Substitute Solution  
 74  
 density 15

- development 68  
 DIPSO buffer 38  
 dynamic fluid environments 197–206  
 efficacy 26  
 equilibration of pH 145, 150, 151  
   in incubators 149, 149–50  
   oil overlay 151–2, 152  
   re-equilibration 150, 150–1  
 fluid dynamics 198–206  
   analytical tools 205–6  
   Braille displays 203–4, 204  
   controlled 203–5  
   microchannels 203, 204  
   microfluidics 203–4, 237  
   microfunnels 204, 204–5  
   platform integration 205–6  
   stimulation through vibration 201–3  
 glutamine content 105–6  
 GM-CSF addition 122, 124  
 growth factors in 112–13  
 HEPES buffer 37–8, 55  
 intact system usage 236  
 interactions 4–16  
 macromolecular components 23–5, 55–6  
 macromolecular supplementation 68–77  
 methionine content 104  
 microchannels 203, 204  
 microfluidics 203–4, 237  
 microfunnels 204, 204–5  
 monoculture 236  
 optimizing systems 241–2  
 osmolality 58, 132–9  
   human media 134  
   maintenance 239–40  
 osmolarity 134  
 pH  
   control 142–3, 148–53, 238, 239  
   stability 237–9  
 plasma volume expanders 72–3  
 pressure gradients 203–4  
 protein-free solutions 69  
 shear stress 198–9, 201  
 static environment 197  
   dynamic fluid environments adapted from 200–1, 202  
 stressor reduction 235  
 systems 235  
 thermal control 236–7  
 vibration 201–3, 202  
 whole serum use 69–71  
 embryo–endometrial dyssynchrony 85–6  
   advanced endometrium 87–8  
   compared with normal communication 87  
   embryo transfer timing 87  
   monozygotic twinning rate 91  
 embryonic stem cell cultures 102–3  
 embryotrophic signals, putative 24–5  
 endometrium  
   advanced 86–9  
   cytokine synthesis 115–18  
   epithelial cell 115–17  
   leukocytes 117–18  
 energy substrates, media 7–9  
 eosinophils 117–18  
 epidermal growth factor (EGF) 162–4  
 epigenetic effects of ART 62  
 ESHRE Task Force on Developing Countries 245–6  
 ESHRE working group  
   interaction with ART companies 278  
 essential amino acids (EAA) 50–2, 95–7  
   culture media for oocyte maturation 159  
   embryo culture media 98–9  
 estradiol, culture media for oocyte maturation 162  
 estrous cycle, tubal fluid influence 23  
 ethylenediaminetetraacetic acid (EDTA) 55  
 Europe  
   CE certification process 187–9  
   international regulation of media 185–93  
   Medical Device Directive 185–90  
 European Medicines Agency 190  
   committees 190  
   submission process/timelines 192–3  
 Fallopian tubal fluid collection 22–3  
   osmolality 134–5  
   *see also* HTF medium  
 Fallopian tubal occlusion 245  
 fatty acids, media content 12  
 Federal Food Drug & Cosmetic (FD&C) Act (USA) 193  
 female tract environment 21–6  
   fluid collection/analysis 22–3  
   media composition 21  
 fertility treatment 211–12  
   *see also* assisted reproductive technologies (ART); *named procedures*  
 fetal programming, GM-CSF 123  
 fluid dynamics 198–9  
   microfluidics 200, 237  
   embryo culture media 203–4  
   sperm sorting 201  
   shear stress 198, 198–9, 201  
   biological resilience to 199  
 follicle-stimulating hormone (FSH)  
   culture media for oocyte maturation 160–1  
   priming for in vitro maturation of human immature oocytes 157  
 folliculogenesis, oocyte growth 157  
 Food and Drug Administration (FDA) 193  
 Forma big-box incubators 51  
 fungal growth risk in incubators 272  
 gametes  
   cryopreservation 38–41  
   encapsulated 252  
 gas analyzers 267, 273  
 gas concentration control in incubators 273  
 gas phase of media 14–15  
 gentamicin 14  
 glassware, washing 6  
 globulins *see* alpha globulin; beta globulin  
 glucose  
   culture media for oocyte maturation 158  
 metabolism 56  
   through Embden–Meyerhof pathway 158  
   vitamin effects 159

- glucose (cont.)  
uptake by embryos 8  
utilization 7
- glutamine 10–11, 50, 52  
ammonia/ammonium  
release in culture media  
105–6  
glycine substitute in media  
137  
preimplantation embryo  
development 99  
uptake by embryo 100
- glutathione (GSH) 53
- glycine 52, 100  
organic osmolyte function  
137–8
- glycoproteins, oviductal 76–7
- glycyl-L-glutamine 10–11, 52  
ammonium release in culture  
media 105
- GLYT1 glycine transporter  
137–8
- gonadotropin-releasing  
hormone (GnRH)  
antagonists 248–9
- gonadotropins  
culture media for oocyte  
maturation 160–1  
use in oocyte retrieval 258
- good manufacturing practice  
(GMP) 194
- Good's buffers *see* BES buffer;  
HEPES buffer; MOPS  
buffer; TES buffer
- granulocyte-macrophage  
colony-stimulating  
factor (GM-CSF) 13
- blastocyst development  
118–19, 123
- in embryo culture media 122,  
124
- embryo development 122–4  
embryo protection from cell  
stress/apoptosis 123–4  
embryotoxicity 118–19  
embryotrophic role 122–3  
fetal programming 123  
secretion 122
- growth factors  
culture media for oocyte  
maturation 160, 162–4  
embryo culture media  
112–13  
embryo development 112–14  
media content 13  
receptor binding 113
- heating, culture dishes 260–1
- heat shock proteins 123–4
- Henderson-Hasselbalch  
equation 54, 54
- heparin-binding epidermal  
growth factor (HB-EGF)  
119
- HEPES buffer 32  
cryopreservation 39–40  
embryos 37–8, 55  
MOPS combination 34, 38  
oocytes 33–4  
sperm 36
- Hoffmann modulation contrast  
imaging unit 214
- hormonal stimulation 248–9
- HTF medium 6, 48, 51, 73–4
- human chorionic gonadotropin  
(hCG) 156
- human follicular fluid (HFF)  
160
- human peritoneal fluid (HPF)  
160
- human serum albumin (HSA)  
55–6, 71  
addition to cryopreservation  
embryo culture media  
74  
alpha and beta globulin  
addition 73–4
- Human Tubal Fluid medium  
*see* HTF medium
- humidity in incubators 261  
control 272
- ice blockers 174
- image acquisition systems in  
incubators 214–15  
dark-field imaging unit with  
software-based embryo  
evaluation 215  
Hoffmann modulation  
contrast imaging unit  
214–15  
integrated TLI unit 215–16
- in vitro fertilization (IVF)  
culture systems 235  
fluid dynamics application in  
laboratory 199–206  
sperm preparation  
199–200  
laboratory environment 235  
air quality 241  
laboratory equipment  
temperature profile  
236–7, 237
- laboratory system utilization  
235
- low cost 245–55  
complications following  
oocyte retrieval 252
- encapsulated gametes 252
- experience in Arusha,  
Tanzania 253–5, 254
- hormonal stimulation  
248–9
- intrauterine insemination  
247–8
- intravaginal culture 252
- laboratory techniques/  
equipment 249–52
- long shelf life media 254
- media 250
- ovarian hyperstimulation  
syndrome 252
- ovarian stimulation 248–9,  
249
- pregnancy complications  
253
- risk minimizing 252–3
- submarine incubators  
250–2
- take-home baby rate 250
- training of professionals  
246–7, 253
- product classification as  
medical devices 186
- regulation history 183
- in vitro fertilization/  
intracytoplasmic sperm  
injection (IVF/ICSI)  
4, 5
- in vitro maturation (IVM) of  
human immature  
oocytes 155–65
- culture media 157–60  
antioxidants 159–60  
composition 163  
conditions 157  
energy sources 158  
gonadotropins 160–1  
growth factors 162–4  
nitrogen sources 159  
proteins 160  
steroids 162  
supplements 160–4  
vitamins 159
- first successful births 156
- follicle size 157
- follicle-stimulating hormone  
priming 157
- historical aspects 155–7

- human chorionic gonadotropin priming 157
- human cumulus–oocyte complex 161
- system development 164–5
- incubators 15, 142, 258–68
- air quality 262
- control 270–2
- altitude 267
- bacterial growth risk 272
- buffers in media 42
- built onto existing microscopes 214
- carbon dioxide levels 261, 273
- monitoring 147, 148, 148–9
- carbon dioxide sensors 266–7
- contact heat/small chamber 265
- culture system 260–2
- design 274
- electromagnetic waves 273–4
- embryo culture media
- equilibration 149–50
- re-equilibration 150–1
- embryo observation systems 265
- evolution 259–60
- Forma big-box 51
- front-loading chamber 263, 274
- fungal growth risk 272
- gas analyzers 267, 273
- gas composition 261–2
- gas concentration control 273
- historical aspects 258–9, 259
- human embryo culture 148–9
- humidity 261
- control 272
- hybrid models 265
- infrared sensors 267
- management 270–5
- media development 50
- micro-incubators 237
- models 263–5
- natural 266
- options 264
- oxygen levels 261–2
- oxygen sensors 266
- particle counts 270–1
- pH 261–2
- pregnancy rate variation 274
- size 260
- submarine 250–2, 251
- technology challenges 266–7
- temperature control 237, 260–1
- temperature profile 236–7
- thermal conductivity 267
- time-lapse imaging 237
- flexible modified standard 215
- videography 15
- top-loading 274
- benchtop 264
- types 260
- volatile organic compounds 271–2
- water pans 272
- see also* image acquisition systems in incubators
- infertility
- care implementation 246–52
- developing countries 245–52
- diagnostic hysteroscopy 247
- experience in Arusha, Tanzania 253–5, 254
- fertility treatment 211–12
- intrauterine pathology 247
- serodiscordant couples 247–8
- tubal occlusion 245
- infrared sensors 267
- inorganic ions, media content 6–7
- inositol 159
- insulin-like growth factor I (IGF-I) 119
- international regulation of media 183–95
- challenges 184–5
- classification 184–5
- Europe 185–93
- CE certification process 187–9
- classification 185–7
- Medical Device Directive 185–90
- history of ART regulation 183
- history of media regulation 183
- Italian law 277
- in major markets 185
- US 193–5
- intracytoplasmic sperm injection (ICSI)
- fluid dynamics application 199–200
- polar body extrusion 213
- pronuclear formation in oocytes 213
- intrauterine insemination (IUI) 247–8
- intravaginal culture 252, 266
- ISolate density gradient material 58
- isoleucine 50
- Krebs cycle 7–8
- Krebs–Ringer Bicarbonate (KRB) 6
- Krebs–Ringer solution 1–2
- KSOM culture medium 133–4
- lactate
- culture media for oocyte maturation 158
- metabolism 57
- large calf syndrome 70, 120
- large offspring syndrome 277
- lectins, zona pellucida penetration 75
- “let the embryo choose” approach 2
- leucine 11
- leukemia inhibitory factor (LIF) 119
- leukocytes, endometrial/oviductal 117–18
- lipoate 53
- Low Cost IVF Foundation 245–6
- lutinizing hormone (LH) 160–1
- lymphocytes, cytokine production 117
- M91 medium 48–50
- macromolecules
- embryonic microenvironment modulation 77
- media content 23–5, 55–6
- organic 56
- penetration of zona pellucida 75–7
- supplementation of embryo culture media 68–77
- see also* albumin; alpha globulin; beta globulin
- magnesium ions, media content 7

- mast cells, uterine 117–18
- media
- amino acids
    - addition 6
    - content 9–12, 51–2
  - ammonium ions 52–3
  - antibiotics 14
    - classification 186–7
  - antioxidants 13, 53
  - bicarbonate ion content 7
  - birthweight of offspring 26
  - calcium ion content 7
  - carbon dioxide levels 61
  - chelators 13
  - chemical composition 51–7
  - commercialization issues 278
  - composition and female tract environment 21
  - contact supplies 15
  - density 57–8
  - development 1–2, 47–62
    - in vivo environment 48
  - early formulation work 48–51
  - EDTA 55
  - embryo density 15
  - embryo interactions 4–16
  - energy substrates 7–9
  - evaporation 261
  - external pH (pH<sub>e</sub>) 30
    - oocytes 33
  - fatty acid content 12
  - formulations 4–6
  - gas phase 14–15
  - glucose content 56
  - growth factors 13
  - inorganic ions 6–7
  - long shelf life 254
  - low cost IVF 250
  - macromolecular components 23–5, 55–6
  - magnesium ion content 7
  - monoculture systems 236
  - novel for bovine zygote culture 23–5
  - nucleic acid precursors 12–13
  - oil overlay 14, 272
  - one-step 61–2
  - optimizing conditions 235–42
  - optimizing systems 241–2
  - osmolality maintenance 239–40
  - osmolarity 58–9
  - osmolytes 57
  - oxidative stress 53
  - pH 59–61
    - measurement variations 60
    - stability 237–9
  - phosphate content 54
  - phosphate removal 6
  - physicochemical
    - characteristics 57–61
  - pyruvate content 57
  - quality control 15–16
  - regulation 183–95
    - challenges 184–5
    - classification 184–5
    - European 185–93
    - history 183
  - renewal 11
  - sequential 48, 61–2
  - tubal fluid 22–3
  - vaginal incubation 252
  - vitamin addition 50–1
  - vitamin content 12
  - water 6
    - see also* cryopreservation
    - media; embryo culture
    - media; in vitro
    - maturation (IVM) of human immature oocytes, culture media
- Medical Device Directive (EU) 185–90
- MES buffer 37
- metabolomics 277
- preimplantation embryo assessment 206
- methionine 50
- embryo culture media 104
  - embryo development
  - epigenetic regulation 103, 103–4
  - fetal development negative impact 104
  - neural tube defects 103–4
  - transporters 104
- microchannels 203, 204
- microfluidics 200, 203–4, 237
  - sperm sorting 201
- microfunnels 204, 204–5
- micro-incubators 237
- Millipore reverse osmosis 6
- Milliq treatment system 6
- mitochondria, preimplantation development 8
- modified mouse embryo assay, morphokinetics 14
- monoamniotic twins 80
- monochorionic, diamniotic (MC-DA) placentation 81, 85–6, 89
- monochorionic placentation 82, 84, 89
- monozygotic triplets 82–3
- monozygotic twinning 80–92
  - advanced endometrium 89
  - analysis 83–4
  - armadillo studies 84–5
  - blastocyst(s)
    - culture condition optimization 91
    - splitting 85, 86
  - blastocyst transfer 82, 84, 90
  - co-culture 90
  - timing 88–9
- causes 81
- cleavage stage transfer 88
- embryo transfer timing 86–7, 91
- embryo–endometrial dyssynchrony 91
- extended embryo culture 91
- genetic component 87, 91
- incidence 80–2, 82
- induced ovulation 88
- laboratory adverse events 90
- model 85–8
- ovarian stimulation length 83–4, 90
- ovulation induction 88, 91
- pathogenesis 85
- placentation 84, 81
  - type 81, 83
- polyembryony 85
- progesterone levels 89
- rate 83
- risk factors 83
- timing of event 82
- MOPS buffer 32
  - cryopreservation 39–40
  - embryos 38, 55
  - HEPES combination 34, 38
  - oocytes 34
  - sperm 36
- morphokinetics
  - embryo monitoring 15
  - modified mouse embryo assay 14
- mouse embryo assay, quality control 15–16
- mouse zygotes, in vivo generated 47
- MUC1 mucin 76, 76
- multinucleation 222–3, 223



- N*-acetyl-glutamine 105
- neural tube defects, methionine  
function 103–4
- neutrophils 117–18
- nitrogen  
culture media for oocyte  
maturation 159  
*see also* amino acids;  
ammonia
- non-essential amino acids  
(NEAA) 51–2, 95–7  
culture media for oocyte  
maturation 159  
embryo culture media 98–9  
energy sources 100
- nucleic acid precursors, media  
content 12–13
- oil overlay  
media 14  
humidity control 272  
pH control of embryo culture  
media 149, 152  
equilibration with 151–2
- oocytes  
amino acids  
in development 95  
transport 97–8  
buffers 32–5  
cAMP 162  
formation 155  
growth during  
folliculogenesis 157  
HEPES buffer 33–4  
incubation requirement 258  
manipulation 240  
maturation 155  
MOPS buffer 34  
origin 22  
oxidative damage 53  
pH stability 237–9  
pronuclear formation in 213  
retrieval 254  
complications following  
252  
gonadotropin use 258  
shear stress sensitivity 240  
temperature sensitivity 236–7  
TES buffer 34  
*see also* in vitro maturation  
(IVM) of human  
immature oocytes
- osmolality 58, 132–9  
definition 132–3  
embryo culture media 58,  
132–9
- cell volume regulation  
136–8  
composition 138  
evaporation 261  
human 134, 138–9  
lowering 136–7  
maintenance 239–40  
optimal 138–9  
Fallopian tube fluid 134–5  
oviduct 134–5
- osmolality 58–9, 133  
embryo culture media 134
- osmolytes 57, 133  
amino acids 57, 99–100  
in culture media for oocyte  
maturation 159  
organic 136  
glycine actions 137–8
- ovarian follicle, environmental  
temperature 25
- ovarian hyperstimulation  
syndrome (OHSS)  
252
- ovarian stimulation  
length and monozygotic  
twinning 83–4, 90  
low cost IVF 248–9, 249
- oviduct 197  
cytokine synthesis in  
leukocytes 117–18  
embryo autonomy 25–6  
environment  
abnormal 26  
temperature 25  
epithelial cell production of  
cytokines 115–17  
epithelium 26  
fluid environment 197  
function 26  
gene expression 26  
glycoprotein penetration of  
zona pellucida 76–7  
GM-CSF secretion 122  
lumen temperature 26  
osmolality 134–5  
physical environment 25  
protein expression 26  
*in situ* animal model 23  
sperm storage/movement  
regulation 26  
zygote development into  
blastocyst 21
- oviduct fluid 26  
estrous cycle influence 23
- ovulation, natural 86  
ovulation induction 86
- monozygotic twinning 88, 91  
*see also* ovarian stimulation
- oxidative stress 53
- oxygen  
altitude effects 267  
concentration in culture  
media for oocyte  
maturation 159  
gas phase 14–15  
incubator levels 261–2
- oxygen sensors 266
- particle counts, incubator air  
quality 270–1
- penicillin 14
- Percoll density gradient  
material 58
- pH  
altitude effects 146–8  
definition 143–4  
incubators 261–2  
measurement 144–6, 145, 153  
factors affecting readings  
146–8  
temperature effects 146
- pH control of embryo culture  
media 59–61, 142–3,  
148–53  
bicarbonate ions 143  
buffers 143, 152–3, 258  
carbon dioxide levels 143–4  
altitude effects 146–8  
determinant of functioning  
culture system 142  
development success 239  
equilibration 145, 149,  
149–50, 150  
oil overlay 151–2, 152  
re-equilibration 150,  
150–1  
maintenance 143, 153  
measurement 145, 145–6,  
152, 153  
variations 60
- oil overlay 149  
equilibration with 151–2,  
152  
optimal range 143  
quality indicator 142  
stability 237–9
- pH, external of media (pH<sub>e</sub>) 30,  
59–61
- oocytes 33
- pH, intracellular (pH<sub>i</sub>) 59–61  
amino acids in regulation  
100

- pH meters 144, 146, 153  
 phenol red 50–1  
 phenylalanine 50  
 phosphate  
 media content 54  
 removal from media 6  
 phosphate buffered solutions (PBS) 33, 35  
 cryopreservation 39–40, 54  
 phosphofructokinase 7  
 PIPES buffer 34, 36  
 placentation  
 armadillo studies 85, 89  
 dichorionic 84, 89  
 monochorionic 82, 84, 89  
 monochorionic, diamniotic (MC-DA) 81, 85–6, 89  
 monozygotic twinning 81, 81, 83, 84  
 plasma protein fractions (PPF) 72–3  
 plasma volume expanders 72–3  
 Plasmanate 72, 74  
 Plasmatein 72  
 polyembryony 85  
 polyglycerol 174  
 polyvinyl alcohol (PVA) 56  
 low molecular weight 174  
 polyvinylpyrrolidone (PVP) 160  
 pregnancy  
 complications 253  
 cytokines and maternal tract  
 quality control 121–2  
 multiple 253  
 rate variation with incubator 274  
 pregnancy rates, IVF/ICSI 4, 5  
 preimplantation development 7–8  
 amino acids consumption/excretion 11  
 prion diseases, transmission  
 risk with albumin use 72  
 progesterone  
 culture media for oocyte maturation 162  
 levels in monozygotic twinning 89  
 proline 102–3  
 pronucleus, formation/fading 220, 226–7, 228  
 proteins  
 amino acids as substrates 96, 101  
 culture media for oocyte maturation 160  
 PureSperm density gradient material 58  
 pyruvate 7, 11  
 antioxidant function 13  
 culture media for oocyte maturation 158  
 metabolism 57  
 storage 8–9  
 quadruplets, armadillo studies 84  
 quality control  
 media 15–16  
 mouse embryo assay 15–16  
 water testing 6  
 quality indicators (QI), pH  
 control of embryo culture media 142  
 quiet embryo hypothesis 56  
 reactive oxygen species (ROS) 13, 53  
 cell death 159  
 developmental arrest 159  
 sperm damage 53  
 recombinant human albumin (rHA) 72  
 regulation *see* CE certification  
 process in Europe;  
 Europe; international  
 regulation of media;  
 USA  
 S-adenosyl methionine (SAM) 103  
 semen, microbial  
 contamination 14  
 semi-permeable membrane 132–3  
 serodiscordant couples 247–8  
 serum *see* whole serum  
 serum albumin  
 culture media for oocyte maturation 160  
 use in embryo culture media 70, 71–2  
 Serum Substitute Solution (SSS) 72–4  
 albumin addition 73–4  
 cryopreservation embryo culture media 74  
 culture media for oocyte maturation 160  
 shear stress 198, 198–9, 201  
 biological resilience to 199  
 embryo/oocyte sensitivity 240  
 sodium bicarbonate  
 buffers 30  
 concentration 42  
*see also* bicarbonate ions  
 SOF embryo culture medium 23, 24, 48  
 sperm/sperm preparation  
 buffers 35–7  
 fluid dynamics application 199–200  
 HEPES buffer 36  
 lactate content of media 57  
 low cost IVF 249–50  
 MOPS buffer 36  
 osmolarity of washing media 59  
 reactive oxygen species  
 damage 53  
 sorting with microfluidic device 201  
 TES buffer 36  
 Tris buffer 35  
 steroids, culture media for  
 oocyte maturation 162  
 STF medium 48, 49  
 streptomycin 14  
 stressors, reduction in embryo culture 235  
 Synthetic Oviduct Fluid medium *see* SOF  
 embryo culture medium  
 Synthetic Serum Substitute (SSS) 72–3, 160  
*see also* Serum Substitute Solution (SSS)  
 TAPSO buffer 34–5  
 taurine 52–3  
 embryo development 99  
 TES buffer 34, 36, 38  
 cryopreservation 41  
 TEST buffer 34–6  
 threonine 102  
 time-lapse imaging (TLI) of embryos 211–30  
 annotation parameters 223  
 clinical benefits 229  
 controlled incubation environment 213  
 differences between available systems 216  
 division cycles 225

- flexible with modified  
standard incubator 215
- incubators 237
- integrated unit 215–16
- light exposure 216, 217
- monitoring system certified  
for clinical use 215
- multinucleation 222–3
- stages of human  
development 212
- strategy adaptation 228–9
- videography in incubation  
chamber 15
- time-lapse monitoring (TLM)  
213, 215–18
- annotation parameters 223
- blastomere size/  
fragmentation 222
- early cleavage 219–21
- light dose 216
- novel selection/de-selection  
parameters 223–9
- safety 216–18  
*versus* standard assessment  
218–23
- T-lymphocytes 117
- Toll-like receptors 116–17
- tricarboxylic acid (TCA) cycle  
7–8
- tricine buffer 37
- triplets 82–3
- Tris buffer 34–5
- Tris:citrate buffer 35
- tubal fluid *see* Fallopian tubal  
fluid
- tubal occlusion 245
- twins 80  
*see also* monozygotic  
twinning
- TYB buffer 36
- USA  
approval process 193–5  
classification  
of ART media 193  
of devices 194
- Code of Federal Regulations  
193
- FDA 193
- good manufacturing practice  
194
- Marketing Clearance 194
- Premarket Notification/  
Premarket Approval  
194
- regulation of media 193–5
- Traditional 510(k)  
notification 194–5
- uterine embryo transfers 85
- uterine fluid, estrous cycle  
influence 23
- uterus  
GM-CSF secretion 122  
mast cells 117–18  
pathology in infertility 247  
Toll-like receptor expression  
by epithelial cells 116–17
- vaginal incubation of media  
252, 266
- vitamin(s)  
addition to media 50–1
- culture media for oocyte  
maturation 159  
glucose metabolism effects 159  
media content 12
- vitamin C 53
- vitamin E 53
- vitrification 173, 180
- vitrification solutions 178, 179
- volatile organic compounds  
(VOC) 271–2
- warming surfaces, temperature  
profile 236–7
- water 6
- Whitten's medium 6
- whole serum  
abnormally large offspring 70  
components 160  
contraindications for use  
70–1  
culture media for oocyte  
maturation 160  
use in embryo culture media  
69–71, 70
- zona pellucida  
macromolecule penetration  
75–7  
shear stress 198  
structure 198
- zwitterions 30–2, 35, 55  
aliphatic amines 30, 32
- zygotes  
development into blastocysts  
in oviduct 21  
in vivo-generated mouse 47