Examples of How Cloning of Mammals Has Developed Over the Years

1. Breeding/Cloning:
   - Protocol: e.g., multiple prize sow ova, artificial fertilization prime boar, divide at 2 cell stage (clones) = many identical twins
   - Benefits: more efficient than selective breeding
   - Shortcomings: don't know phenotype in advance—maternal-paternal genomes coming together—inefficient

2. Nuclear transfer using embryonic stem cells (ESC):
   - Protocol: harvest eggs and blastocysts, isolate inner cell mass (ICM=ESC) cells, isolate ESC nucleus, transplant nucleus into enucleated egg, culture zygote in vitro to blastocyst stage, implant into uterus
   - Benefit: can get more than 2 of the same
   - Shortcomings: limited by number of blastocyst stage embryos and eggs obtainable, harvesting ICM, nuclear transfer, don't know phenotype in advance

3. Nuclear transfer using cultured ESC:
   - Protocol: harvest blastocysts, dissociate ICM cells, culture ESC with a cytokine (leukemia inhibition factor) to block spontaneous differentiation into "embryoids", ESC nucleus transfer to enucleated egg, culture zygote in vitro to blastocyst stage, implant into uterus.
   - Benefit: continuous supply of same phenotype ESC
   - Shortcomings: collecting eggs, nuclear transfer, don't know phenotype in advance

4. Fusion of cultured ESC with enucleated egg:
   - Protocol: #3 protocol but fuse cultured ESC with egg using an electrical pulse (sticky pms) and a second pulse to activate "zygote" metabolism instead of nuclear transfer
   - Benefit: eliminates nuclear transplantation
   - Shortcomings: like #3 except nuclear transfer, don't know phenotype in advance

5. ADULT cell or adult cell nucleus with enucleated egg:
   - Protocols: How Dolly and Cumulina were made
   - Benefits: only need eggs and adult cells, know phenotype in advance
   - Shortcomings: technical efficiency, consistent reprogramming