Species-specific primary cell cultures: a research tool in veterinary science

Karim R. Sultan and Henk P. Haagsman

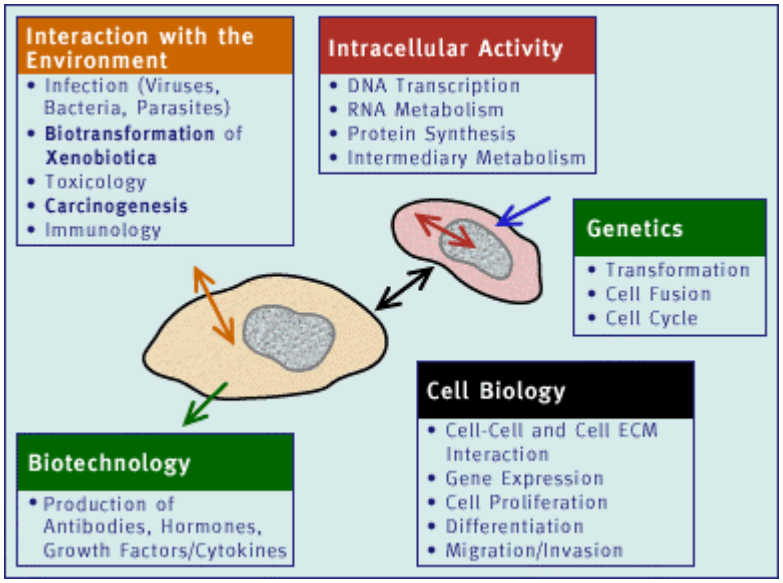
Department of the Science of Food of Animal Origin, Faculty of Veterinary Medicine, Utrecht University, PO Box 80.175, NL-3508 TD, Utrecht, The Netherlands.

Introduction

In experimental veterinary research intact animals are often employed. Although this will remain important, both basic and applied research may benefit from well-chosen and well-designed model systems, which range from isolated perfused organs to subcellular fractions. Cell and tissue cultures of organs of euthanised companion animals and slaughtered production animals have been used only infrequently in veterinary science. However, like no other method, cell culture systems offer possibilities to screen for effects of compounds like hormones and drugs in a controlled way and under a wide variety of conditions. Application of new technologies commonly termed "functional genomics" will help to identify (cell-specific) target molecules. Thus, cell culture systems may contribute considerably to knowledge in the veterinary sciences. Here is a brief overview of the potential of species-specific primary cell cultures.

Isolation of viable cells for culture

Over the last three decades, cell and tissue culture methods have been refined and have now become an essential tool in biomedical research. Animal welfare concerns may recently have played a role in this development, but the main reason was to develop systems that allow the study of single cellular functions under controlled environmental conditions (see Fig. 1).



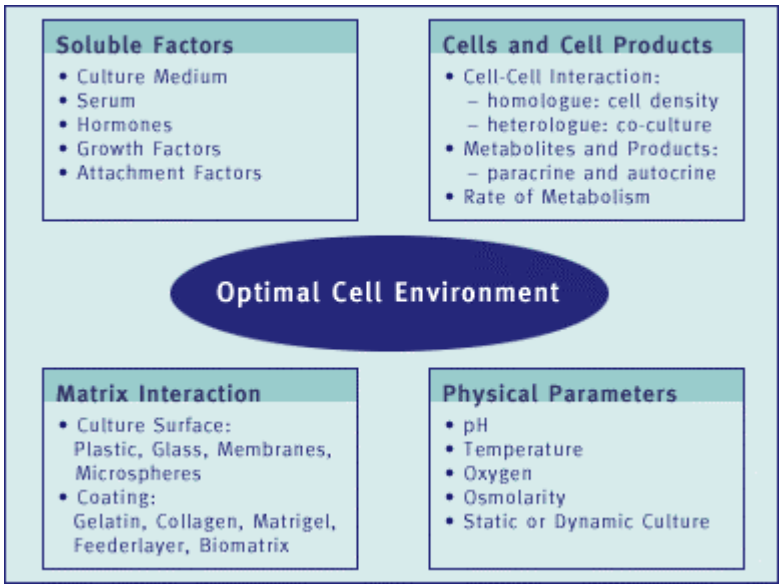
In vitro systems share the characteristic that they exclude the influence of other organs and of the circulatory and immune system, thus providing the possibility to study direct effects on a cell population. Today's cell culture systems are based on mechanical and/or enzymatic disaggregation of the tissue to single cells. Tissue samples are mostly obtained from laboratory animals that are killed for this purpose, and from embryonated chicken eggs. Biopsy specimens or samples from surgically removed material are another source, but their use is limited by irregular availability, small volume, and difficulties in standardisation due to variations in sample origin (genotype, strain/breed, age etc.).

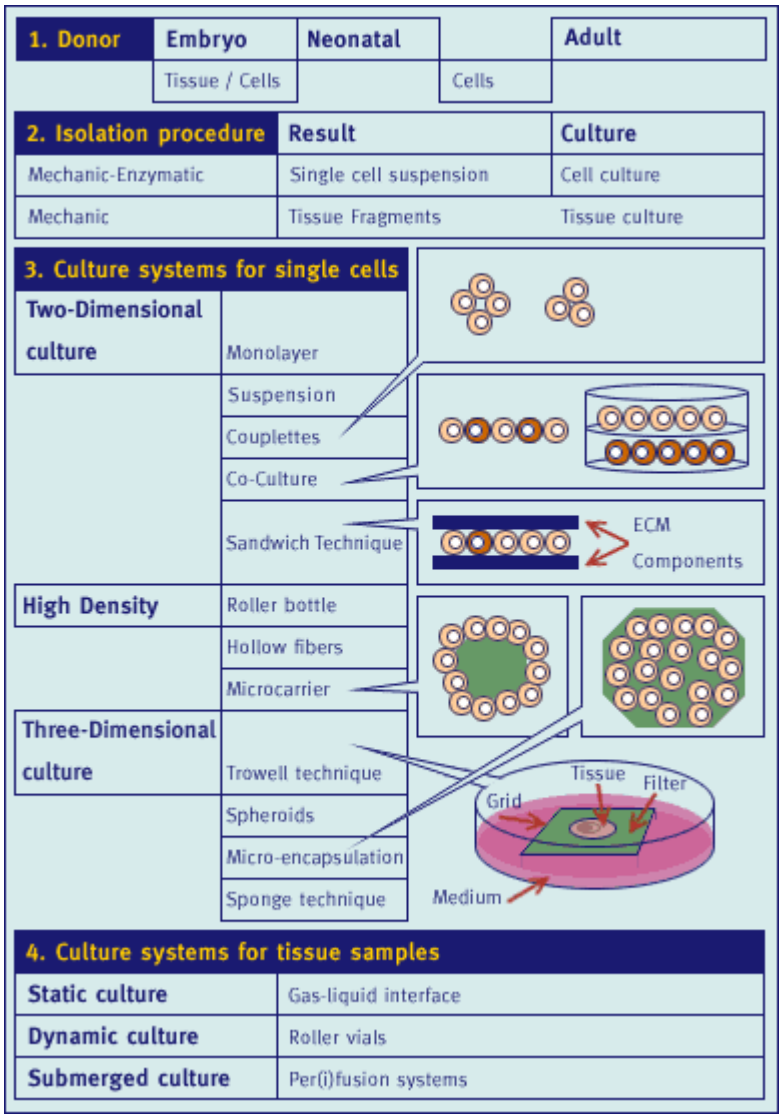
Primary cell cultures vs. cell lines

Two types of cell culture should be distinguished:

1. Primary cultures, which are obtained directly from an animal and can keep the differentiated state for a short period (days to weeks). Functionally differentiated primary cell cultures have a limited life span, and although maintenance of the differentiated properties has been improved by additives to the culture medium, components of the extracellular matrix or by different forms of co-culture (see Figures 2 and 3), cell specific functions will eventually decline.

2. Permanent cultures (e.g. HeLa, 3T3, MDCK), which have an unlimited proliferation capacity and which originated from embryos, tumors or transformed cells





Cells can proliferate and/or differentiate, both with different limitations, depending on the cell type studied (e.g. neurons). Numerous publications provide protocols for the isolation of different cell types, their culture conditions, and for the evaluation of the degree of differentiation. Much attention is presently given to stem cells (see our News section). Primitive pluripotent embryonic stem cells derived from post-mortem foetal tissue have been used in cell therapy studies to replace diseased tissues. Until recently it was difficult to isolate and culture stem cells from adult tissues other than bone marrow. However, the cell therapy field is rapidly expanding, and it appears that many tissues harbour adult stem cells that possess a much greater plasticity than previously thought. Adult stem cells may obviously also be used as a model system, and it is to be expected that they will receive more attention also in the veterinary sciences.

Primary cell cultures from farm animals

When searching through the literature, it is quite easy to find cell culture systems for any desired tissue. However, not all published protocols are accepted as standard procedures, and there is a large variety of culture conditions. In general, care should be taken when

comparing methods and extrapolating a system from one species to the other. There are only a few publications dealing with primary cultures derived from veterinary relevant species (as compared to those from laboratory animals). It was the use of laboratory animals, which has resulted in the development of standard procedures - but these must be adapted for each species. It can be expected that species-specific cell culture systems will become important to complement results obtained by genome and proteome analysis, both in human and in veterinary research.

Nevertheless, primary cell cultures of several tissues have found their way into basic and clinical veterinary research. The choice of animal and organ is often made on practical grounds, like availability of tissue or slaughterhouse procedures. For example, isolation of porcine pneumocytes from slaughterhouse material will be difficult, as many lungs are affected by the scalding procedure. On the other hand, kilograms of bovine adrenals can be collected in the slaughterhouse, from which pure adrenal cortex cells are easily prepared that are viable for some weeks and maintain differentiated functions. Fig. 4 shows bovine adrenal cortex cells cultured for six days in serum-free medium and treated with ACTH, cAMP, dexamethasone, cycloheximide, and tunicamycin.

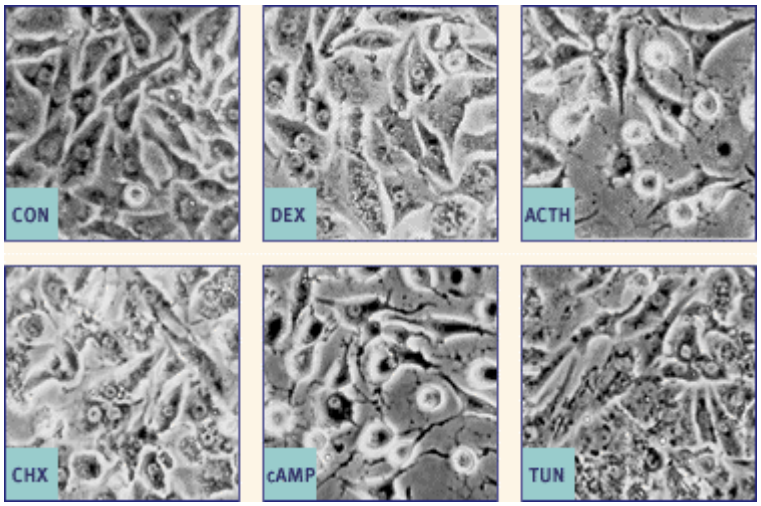


Fig. 4 Exposition of primary bovine adrenal cortex cells to hormones (ACTH and dexamethasone), second messenger (cAMP), and inhibitors of protein translation (cycloheximide) and glycosylation (tunicamycin). Primary cells were incubated for four days in F-12 medium containing 10% fetal calf serum. After proliferation to confluence, cells were cultured in serum-free medium, with the test compounds present for two days. Upper panel left: controls (CON); upper panel right: 50 µM dexamethasone (DEX); central panel left: 1 µM ACTH; central panel right: 10 µg/ml cycloheximide (CHX); bottom panel left: 1 mM cAMP; bottom panel right: 2,5 µg/ml tunicamycin (TUN). Magnification: x200.

Endocrine tissue

For these technical reasons it is not surprising to find high-ranking publications using bovine adrenal cortex cultures [5,10]. The protocols leading to a standardised procedure have been developed in the late 70ies [14, 18]. More information concerning optimal culture conditions of these cells can be found in the literature [9, 24],. Other endocrine tissue has been successfully cultured, like bovine and porcine thyroid cells [2]. A detailed description of cell cultures from porcine pancreas has been published [20]. Primary cell cultures from the porcine reproductive system have also been described, like endometrial cells [6], cells from the ovary [11], and Leydig cells [3, 13]. Bovine mammary epithelial cell culture was employed to study the synthesis and secretion of IGF binding proteins [12]., and bovine pituitary cells for the analysis of signaling pathways [22].

Liver

The isolation of cells from the liver has been a challenge for nearly two decades. The main problem is the rapid onset of cell death post mortem. However, the importance of this

tissue led to large efforts in method development to maintain viable and differentiated cultures from large animals. Bovine, canine and sheep primary hepatocytes were studied for their hormonal responsiveness under serum free conditions [7, 8, 16]. Adult chicken hepatocytes were analysed for their differentiation state under serum free conditions [23]. The optimisation of differentiation was also the focus of studies using porcine hepatocytes [15, 19]. These refinements are of great importance, as improvement of the quality of primary cultures makes them a viable alternative to in vivo experiments, particularly for pre-screening. Thus liver cell culture systems were employed to study aspects of inflammation in bovine hepatocytes [1, 17].

A methological option that needs more attention is the preparation of cell cultures from animals that had died in veterinary clinics. In a recent publication the development of equine adipocyte cultures from expired animals has been described [21].

Skeletal muscle

Skeletal muscle is also easy to obtain and to process into viable cultures. It can be obtained from a variety of animals, and used even many hours post mortem. A recent review [4] summarises isolation and culture protocols from animals of veterinary interest. In the case of skeletal muscle culture it is important to realize that these do not result from outgrowth of existing fibers. It is the dormant stem cells, satellite cells, which are isolated and which fuse in vitro to form new myotubes.

It has been the aim of this mini-review to briefly summarize the principles of primary cell culture and to show that protocols are available for various species of veterinary relevance. The above list of tissues as a source of primary cell culture is by no means exhaustive; cartilage, bone, skin, kidney, spleen, blood derived cells, lung and intestinal tissues have also successfully been processed for primary cell cultures.

Future Developments

The development and standardisation of species-specific primary cell cultures will likely become more important in veterinary research. We can expect to get new insights while exploring and modulating metabolism and function at the cellular level. The diversity of the physiological and pathological features of companion and farm animals is guaranteed to reveal the equivalent on the cellular level. Perhaps the best feature of primary cell cultures is the possibility to modulate the metabolic and regulatory pathways of cells of interest and to delineate the physiological effects of various compounds and drugs in a controlled way. The refinement of this experimental tool can be expected to accelerate species-specific research. Furthermore, cell cultures can be employed as screening systems in various fields of veterinary science, thereby reducing the need for live animals.

References

1. Alsemgeest SP, van 't Klooster GA, van Miert AS, Hulskamp-Koch CK, Gruys E. Primary bovine hepatocytes in the study of cytokine induced acute-phase protein secretion in vitro. Vet Immunol Immunopathol. 1996 Sep;53(1-2):179-84.

2. Bocanera LB, Aphalo P, Pisarev MA, Gartner R, Silberschmidt D, Juvenal GJ, Beraldi G, Krawiec L. Presence of a soluble inhibitor of thyroid iodination in primary cultures of thyroid cells. Eur J Endocrinol. 1999 Jul;141(1):55-60.

3. Brun HP, Leonard JF, Moronvalle V, Caillaud JM, Melcion C, Cordier A. Pig Leydig cell culture: a useful in vitro test for evaluating the testicular toxicity of compounds. Toxicol Appl Pharmacol. 1991 Apr;108(2):307-20.

4. Burton NM, Vierck J, Krabbenhoft L, Bryne K, Dodson MV. Methods for animal satellite cell culture under a variety of conditions. Methods Cell Sci. 2000 Mar;22(1):51-61.

5. Danik M, Chinn AM, Lafeuillade B, Keramidas M, Aguesse-Germon S, Penhoat A, Chen H, Mosher DF, Chambaz EM, Feige JJ. , Protein, Nucleotide Bovine thrombospondin-2: complete complementary deoxyribonucleic acid sequence and immunolocalization in the external zones of the adrenal cortex. Endocrinology. 1999 Jun;140(6):2771-80.

6. Davis DL, Blair RM. Studies of uterine secretions and products of primary cultures of endometrial cells in pigs. J Reprod Fertil Suppl. 1993;48:143-55. Review.

7. 7: Donkin SS, Armentano LE. Preparation of extended in vitro cultures of bovine hepatocytes that are hormonally responsive. J Anim Sci. 1993 Aug;71(8):2218-27.

8. Emmison N, Agius L, Zammit VA. Regulation of fatty acid metabolism and gluconeogenesis by growth hormone and insulin in sheep hepatocyte cultures. Effects of lactation and pregnancy. Biochem J. 1991 Feb 15;274 (Pt 1):21-6.

9. Feige JJ, Keramidas M, Chambaz EM. Hormonally regulated components of the adrenocortical cell environment and the control of adrenal cortex homeostasis. Horm Metab Res. 1998 Jun-Jul;30(6-7):421-5. Review.

10. Gaillard I, Keramidas M, Liakos P, Vilgrain I, Feige JJ, Vittet D. ACTH-regulated expression of vascular endothelial growth factor in the adult bovine adrenal cortex: A possible role in the maintenance of the microvasculature. J Cell Physiol. 2000 Nov;185(2):226-34.

11. Gangrade BK, May JV. The production of transforming growth factor-beta in the porcine ovary and its secretion in vitro. Endocrinology. 1990 Nov;127(5):2372-80.

12. Gibson CA, Staley MD, Baumrucker CR. Identification of IGF binding proteins in bovine milk and the demonstration of IGFBP-3 synthesis and release by bovine mammary epithelial cells. J Anim Sci. 1999 Jun;77(6):1547-57

13. Goddard I, Bouras M, Keramidas M, Hendrick JC, Feige JJ, Benahmed M. Transforming growth factor-beta receptor types I and II in cultured porcine leydig cells: expression and hormonal regulation. Endocrinology. 2000 Jun;141(6):2068-74.

14. Gospodarowicz D, Ill CR, Hornsby PJ, Gill GN. Control of bovine adrenal cortical cell proliferation by fibroblast growth factor. Lack of effect of epidermal growth factor. Endocrinology. 1977 Apr;100(4):1080-9.

15. Gregory PG, Connolly CK, Toner M, Sullivan SJ. In vitro characterization of porcine hepatocyte function. Cell Transplant. 2000 Jan-Feb;9(1):1-10.

16. Hadley SP, Hoffmann WE, Kuhlenschmidt MS, Sanecki RK, Dorner JL. Effect of glucocorticoids on alkaline phosphatase, alanine aminotransferase, and gamma-glutamyltransferase in cultured dog hepatocytes Enzyme. 1990;43(2):89-98..

17. Hoebe KH, Monshouwer M, Witkamp RF, Fink-Gremmels J, van Miert AS. Cocultures of porcine hepatocytes and Kupffer cells as an improved in vitro model for the study of hepatotoxic compounds. Vet Q. 2000 Jan;22(1):21-5.

18. Hornsby PJ, Gill GN. Characterization of adult bovine adrenocortical cells throughout their life span in tissue culture. Endocrinology. 1978 Mar;102(3):926-36.

19. Hosagrahara VP, Hansen LK, Beilman GJ, Remmel RP. Evaluation of the effect of culture matrices on induction of CYP3A isoforms in cultured porcine hepatocytes. Chem Biol Interact. 2000 Jun 15;127(1):91-106.

20. Ohgawara H, Shikano T, Fukunaga K, Yamagishi M, Miyazaki S. Establishment of monolayer culture of pig pancreatic endocrine cells by use of nicotinamide. Diabetes Res Clin Pract. 1998 Oct;42(1):1-8.

21. Reedy SE, Powell DM, Williams NM, Dodson MV, Fitzgerald BP. Thoughts on the source of tissue on subsequent cell culture success. Methods Cell Sci. 2000 Mar;22(1):29-32

22. Roh SG, He ML, Matsunaga N, Hidaka S, Hidari H. Mechanisms of action of growth hormone-releasing peptide-2 in bovine pituitary cells. J Anim Sci. 1997 Oct;75(10):2744-8

23. Yamanaka N, Kitani H, Mikami O, Nakajima Y, Miura K. Serum-free culture of adult chicken hepatocytes; morphological and biochemical characterisation. Res Vet Sci. 1997 May-Jun;62(3):233-7.

24. Yonemitsu N. Functional and structural differences between cultured outer and inner layer cells of bovine adrenal cortex. Cell Tissue Res. 1987 Dec;250(3):507-12.