Physiology & Behavior 187 (2018) 2–5

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Applying the new SABV (sex as a biological variable) policy to research and clinical care

Janine Austin Clayton

Office of Research on Women's Health, National Institutes of Health, 6707 Democracy Boulevard, Bethesda, MD 20817, United States

ARTICLE INFO

Keywords:
Sex differences
Policy
SABV
Rigor
Reproducibility

ABSTRACT

Sex as a biological variable (SABV) is a key part of the new National Institutes of Health (NIH) initiative to enhance reproducibility through rigor and transparency. The SABV policy requires researchers to factor sex into the design, analysis, and reporting of vertebrate animal and human studies. The policy was implemented as it has become increasingly clear that male/female differences extend well beyond reproductive and hormonal issues. Implementation of the policy is also meant to address inattention to sex influences in biomedical research. Sex affects: cell physiology, metabolism, and many other biological functions; symptoms and manifestations of disease; and responses to treatment. For example, sex has profound influences in neuroscience, from circuitry to physiology to pain perception. Extending beyond the robust efforts of NIH to ensure that women are included in clinical trials, the SABV policy also includes rigorous preclinical experimental designs that inform clinical research. Additionally, the NIH has engaged journal editors and publishers to facilitate reproducibility by addressing rigor and promoting transparency through scientifically appropriate sex-specific study results reporting. The Sex And Gender Equity in Research (SAGER) guidelines were developed to assist researchers and journal editors in reporting sex and gender information in publications [1].

1. Introduction

To appreciate the consideration of sex as a biological variable, it is necessary to define and distinguish sex from gender. “Sex” originates from an organism's sex chromosome complement—XX or XY chromosomes in humans, and is reflected in the reproductive organs. Each cell has a sex. One's sex affects all aspects of physiological functioning, not just hormonal secretions. Although one's sex can also affect one's behavior, other factors, social and cultural, can also influence behavior. Thus, the term “gender” pertains to social, cultural, and psychological traits linked to human males and females through social context.

2. Why did the NIH initiate a policy of accounting for sex as a biological variable?

In the 1990s, the NIH instituted a policy to ensure the inclusion of women in NIH-funded clinical research, because it became clearer that there were differences in disease manifestation and response to treatment between men and women, and therefore findings from men could not necessarily be applied to women. In the past, women had been excluded from clinical trials for a variety of reasons, from protectionism to paternalism and concerns that women's variable hormonal status could not be adequately controlled for. So, instead, many researchers chose to ignore sex as a variable. Consequently, males dominated animal studies as well as clinical investigations. A study by Beery and Zuker showed that in six fields (general biology, immunology, neuroscience, physiology, pharmacology, and endocrinology) male rodents only were used in 80% or more of animal studies [2].

If researchers assume that people are more alike than they are different, important differences between sexes, races, and exposures will be overlooked. In fact, sex was a variable that was often considered to be negligible and, hence, one that often could be ignored. Although humans share most of the same genes, as well as many in common with animal species, we have learned that there are many more differences between the sexes, at the cellular, molecular, and genetic levels, than just the sex chromosome complement. The more researchers questioned the assumption of sameness and looked for differences, the more they began to find them. The SABV policy is not a mandate to look specifically for sex differences; however, it does require considering the effects of sex as a biological variable at all stages of research, from the development of a research question through the reporting of study results. The rationale behind the policy is the study of both sexes as a guiding principle in biomedicine. SABV policy is concerned with how males and females are both similar and different [3]. Indeed, the underlying paradigm is changing from an “either/or” to “both/and” approach [4].

As a taxpayer-funded institution, the NIH has responsibility to

E-mail address: Janine.Clayton@nih.gov.

http://dx.doi.org/10.1016/j.physbeh.2017.08.012
Received 26 June 2017; Received in revised form 14 August 2017; Accepted 15 August 2017
Available online 17 August 2017

obtain the most complete, unbiased, and reliable information possible. Without knowing how the sexes are alike and different, function similarly or differently, and respond to treatments similarly or differently, our knowledge base is incomplete. Furthermore, the scientific establishment came under criticism a) for not replicating many studies [5] and b) because those that were replicated often failed to reach the same conclusions [6–9]. Thus, a fundamental pillar of science—reproducibility—was buckling, threatening to collapse the entire edifice. To address these serious issues, new policies promoting rigor and reproducibility were instituted, of which the SABV policy is part. "Scientific rigor is the strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation, and reporting of results. This includes full transparency in reporting experimental details so that others may reproduce and extend the findings" [10].

As the NIH's purview pertains to *clinical* trials—i.e., studies involving human participants that are intended to identify how to improve health and treat disease—the lack of study reproducibility, which may *put people at risk of being harmed*, was of great concern. Indeed, several drugs have been recalled because of severe adverse effects in women, which were not discerned by the clinical trials conducted often without sufficient populations of women [11]. These scientific missteps risk an erosion of public trust. Studying both sexes, not only in clinical trials but also in preclinical research, will fill in the gaps in our understanding of female and male biology and inform sex- and gender-appropriate medical care for both women and men. Because the absence of evidence is not evidence of absence, it is incumbent upon researchers to find all the evidence.

Thus, both sex and gender play distinct roles in how health and disease processes differ across individuals, and consideration of these factors in research studies informs the development and testing of preventive and therapeutic interventions.

3. What is the new SABV policy?

The specific policy regarding rigor and reproducibility is reported in NOT-OD-15-103, and the policy regarding SABV in NOT-OD-15-102. In addition, NOT-OD-16-011 addresses "Implementing Rigor and Transparency." All of these notices can be found on nih.gov. The key points of the policies are as follows:

"These new instructions and revised review criteria will focus on four areas deemed important for enhancing rigor and transparency: 1) the scientific premise of the proposed research, 2) rigorous experimental design for robust and unbiased results, 3) consideration of relevant biological variables, and 4) authentication of key biological and/or chemical resources" (see https://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-103.html).

The SABV policy states that the "NIH expects that sex as a biological variable will be factored into research designs, analyses, and reporting in vertebrate animal and human studies" (see NOT-OD-15-102: Consideration of Sex as a Biological Variable in NIH-funded Research https://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-102.html). By studying both sexes, we will also fill in the gaps in the knowledge base. The policies do not require researchers to use any particular method, to double the sample size, or to power studies to detect sex differences.

In short, these new policies reiterate NIH expectations regarding rigor and enjoin researchers to transparent reporting, so that studies can be reproduced.

4. How is the NIH implementing the new policy?

Because scientific progress involves the activities of a system of interdependent organizations, the NIH is addressing the issue of sex and gender inclusion across biomedical research multidimensionally—through program oversight, review, and policy, as well as through collaboration with many stakeholders in the scientific community, including publishers, societies, industry, media, nonprofit organizations, and educational institutions [3]. In addition, the NIH has made resources available online, such as FAQs and videos, to help clarify the policy and suggest study designs, methods, and models to help researchers apply SABV to their field. Guidance on implementing the policy can be found at https://orwh.od.nih.gov/resources/pdf/NOT-OD-15-102_Guidance.pdf.

5. How can SABV be implemented in research and in clinical practice?

5.1. Research

Considering SABV is not the same as looking for sex differences, but it is about exploring the influences of sex as a biological variable and revealing the "data hiding in plain sight." Here, we describe some ways to help researchers "see" potential influences of sex. First, before conducting research, find out whether there are known sex differences in the area of study by adding the terms "sex," "gender," "male," and "female" to your literature search. In addition to PubMed, use the GenderMed database. Second, randomize and balance the sexes in the study and control groups. If you are testing a pharmaceutical, consult the FDA snapshot page, which provides information about sex differences in drug metabolism and effects for recently approved drugs. Third, if sex differences are suspected, e.g., from the literature search, conduct pilot studies to determine whether powering the study to detect sex differences is warranted. Fourth, in the analyses of the data, regardless of whether the study was powered to detect sex differences, disaggregate the data to see if there are differences that are hidden when data from males and females are pooled. Analyze key relationships for males and females separately. Table 1 summarizes some useful study designs and experimental approaches for considering SABV. For more information consult the NIH's online tutorials and courses (for CME credit) at http://orwh.od.nih.gov/resources/cme.asp, and the guidance document at https://orwh.od.nih.gov/resources/pdf/NOT-OD-15-102_Guidance.pdf.

A clear example of the importance of disaggregating the data can be seen in Fig. 1. McCullough et al. studied the nitric oxide pathway in ischemic cell death in mice [12]. Had they combined the results of the

Table 1
Research designs for studying SABV [4].

Research methods	Attributes	References
Single-variable design	Single experiment, single effect	Shaw et al. [34]
Complete/fractional factorial design	Enables more than one independent variable	Collins et al. [35]
Randomized block design	Variability within blocks is less than variability between blocks	Festing [36]
Ancillary variable design	Sex captured but not an independent variable	Collins et al. [35]
4-Core genotype model	Differentiates between gonadal and nonhormonal sex effects (mice only)	De Vries et al. [37]
Hormone depletion/replacement	Identifies hormonal effects without estrous cycling	Greenspan et al. [38]
Sex-hormone receptor knockouts	Whole-animal (mouse) approach to study hormone effects	Kerkhofs et al. [39] Walker et al. [40]

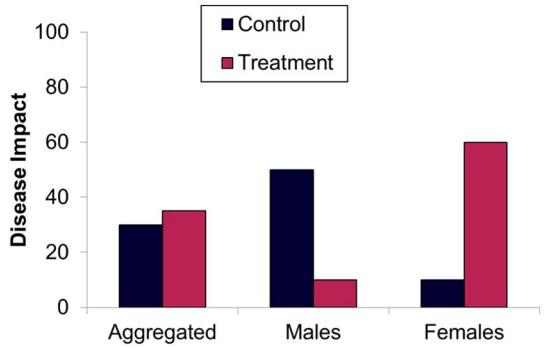

Fig. 1. Schematic diagram showing sex differences in disease impact similar to those reported by McCullough et al. [12]. In cases like this, important sex-by-treatment interactions can be masked when investigators only analyze data sets in which the two sexes are pooled. Disaggregating the data and considering the sexes separately can unmask important sex influences in laboratory animals or human subjects.

male and female mice (Fig. 1, left), they may have found no significant difference between control mice and mice treated with a selective poly-ADP ribose polymerase (PARP-1) inhibitor. By factoring SABV into the design and analysis of the study, it was clear that treatment at onset of ischemia benefitted male mice, but it caused significantly more ischemic damage in female mice (Fig. 1, right). Relationships that approach statistical significance could suggest areas for further follow-up studies in which to look for sex differences. If appropriate, the estrous cycle or specific hormones can be analyzed as mediators, or sex can be controlled for as a confounding variable.

When publishing research, the new SABV policy calls for the reporting of the sex of animals and humans. "Transparency in research calls for accounting fully and accurately for all relevant biological variables—even if they are not elements of the hypothesis. Telling other investigators about the use of females and males enhances the knowledge base for future studies and helps prevent unnecessary and costly duplication of experiments" [4]. To help researchers know what information to include in publications, the SAGER (Sex And Gender Equity in Research) guidelines have been developed (Table 2). In addition to detailed reporting on methods, the guidelines advise researchers to consider the influence of sex in the interpretation of results and to generalize research findings appropriately.

5.2. Clinical care

The SABV policy fosters a systems-based understanding of the influences of sex and gender on health and disease. From sex effects at the level of cells, which contribute to sex influences at the level of systems, which affect sex differences at the level of the individual patient, it is necessary not only to understand the impacts of sex at the basic science level but to apply them, as appropriate, to clinical care. With people, the important roles of gender, race, ethnicity, age, and many other demographic variables must also be factored in, but considering them is beyond the scope of this brief summary of SABV.

How do sex differences in cells inform clinical care? There are known sex differences at the cellular level, for example, in the response to oxidative imbalance [13–15] and in the susceptibility to undergo apoptosis [16]. Under stress, estrogen rescues female bone marrow-derived macrophages from death, whereas in male cells the mechanism of cell death shifts from autophagy to apoptosis [17]. How does this translate to the clinical situation? Male patients undergoing surgery might require different or more aggressive types of neuroprotective treatment.

In the cardiovascular system, gonadal hormones as well as sex chromosomes account for sex differences in ischemic stroke [18]. Sex hormones are known to modulate the immune system as well, resulting in differences in incidence and severity of disease. For example, there is a generally higher prevalence of autoimmune disease in females, particularly with lupus and multiple sclerosis, but some manifestations of those conditions can be more severe in males [19]. For example, lupus-related nephritis is more prevalent in male than female patients [20].

Sex differences in the nervous system include differences in the perception of pain, with female patients reporting more pain in virtually all conditions [21,22]. In addition, differences in the actions of sex hormones suggest differences in how neuroactive agents operate, as well as differences in opiate and nonopiate systems, nerve growth factor, and the sympathetic nervous system. Females seem to have greater perception of pain, being better able to discriminate severity and location of pain.

To provide high quality and safe medical care, it is necessary to know how sex and gender affect each of the following areas:

- *Chief complaint and symptoms*. Men and women experience, process, and report pain differently [21,22]. There are likely to be differences in their experience of other symptomatology.
- *Medical history, including pregnancy history*. Why is pregnancy history important? Consider a patient who is 75 years old and experiencing dizziness. If the patient is a male, of course you would ask about cardiovascular disease history. But if the patient is a 75-year-old female, it would be wise to ask about pregnancy history as well, as a history of pre-eclampsia is a risk factor for stroke later in life.
- *Physical exam, vital signs, lab values, and diagnostic test results*. Some norms for standard lab values differ between males and females; for example, median (2.5–97.5 percentile) growth hormone in males 27–43 years old is 0.16 (0.05–7.5) mIU/L, whereas in females it is 10.9 (0.6–31.0) mIU/L [23,24]. In a recent study [25], plasma melatonin concentration was measured as 642 ± 47 pg/mL/h in males, whereas it was 937 ± 104 pg/mL/h in females taking an

Table 2
Sex And Gender Equity in Research (SAGER) guidelines [1].

General principles
- Authors should use the terms *sex* and *gender* carefully in order to avoid confusing both terms.
- Where the subjects of research comprise organisms capable of differentiation by sex, the research should be designed and conducted in a way that can reveal sex-related differences in the results, even if these were not initially expected.
- Where subjects can also be differentiated by gender (shaped by social and cultural circumstances), the research should be conducted similarly at this additional level of distinction.

Recommendations per section of the article

Title and abstract	If only one sex is included in the study, or if the results of the study are to be applied to only one sex or gender, the title and the abstract should specify the sex of animals or any cells, tissues, and other material derived from these and the sex and gender of human participants.
Introduction	Authors should report, where relevant, whether sex and/or gender differences may be expected.
Methods	Authors should report how sex and gender were taken into account in the design of the study, whether they ensured adequate representation of males and females, and justify the reasons for any exclusion of males or females.
Results	Where appropriate, data should be routinely presented disaggregated by sex and gender. Sex- and gender-based analyses should be reported regardless of positive or negative outcome. In clinical trials, data on withdrawals and dropouts should also be reported disaggregated by sex.
Discussion	The potential implications of sex and gender on the study results and analyses should be discussed. If a sex and gender analysis was not conducted, the rationale should be given. Authors should further discuss the implications of the lack of such analysis on the interpretation of the results.

oral contraceptive.

- *Diseases and conditions*. Cardiovascular disease, Alzheimer's disease, and even eye health show sex differences. Males have higher prevalence of ocular syphilis, acute retinal necrosis, and progressive outer retinal necrosis; yet, two-thirds of visually impaired people are female [26]. Twice as many women have dry eye disease than men [27], and there are more women with age- and estrogen-related cataracts [28].
- *Treatments*. Females metabolize drugs differently and sometimes require lower doses than males. For instance, females clear zolpidem more slowly than do males, resulting in a lower dosage recommendation [29–31]. Drugs may also affect males and females differently. For example, aspirin has cardiovascular protective effects that differ between the sexes [32,33].

6. Conclusion

Sex and gender biomedical research extends far beyond the realm of reproductive health—it spans many diseases and conditions. It starts with differences at the cellular level, which extend through systems to the whole organism, i.e., to patients. Hence, considering the impact of sex at all levels of biology will help expand the knowledge base of male and female biology and inform appropriate individualized care for women as well as men. Accounting for SABV is the first step on the road toward personalized and ultimately precision medicine. To promote the most accurate and comprehensive science possible, the NIH policy to study sex as a biological variable entails that researchers consider sex in their study designs, collect data on both sexes unless there is justification not to, analyze the data accordingly, and report the results completely and transparently. Unless the effects of sex and gender are studied, we will continue to have gaps in the knowledge base, which may result in missed opportunities for discovery and better health. Having greater awareness of the roles that sex and gender play in health and disease will help the entire medical scientific enterprise achieve more clarity and enhance rigor and reproducibility.

References

[1] S. Heidari, T.F. Babor, P. De Castro, S. Tort, M. Curno, Sex and Gender Equity in Research: rationale for the SAGER guidelines and recommended use, Res. Integr. Peer Rev. 1 (2016) 1–9.
[2] A.K. Beery, I. Zucker, Sex bias in neuroscience and biomedical research, Neurosci. Biobehav. Rev. 35 (2011) 565–572.
[3] J.A. Clayton, F.S. Collins, Policy: NIH to balance sex in cell and animal studies, Nature 509 (2014) 282–283.
[4] J.A. Clayton, Studying both sexes: a guiding principle for biomedicine, FASEB J. 30 (2016) 519–524.
[5] M.C. Makel, J.A. Plucker, B. Hegarty, Replications in psychology research: how often do they really occur? Perspect. Psychol. Sci. 7 (2012) 537–542.
[6] M. Baker, 1500 scientists lift the lid on reproducibility, Nature 533 (2016) 452–454.
[7] Open Science Collaboration, Estimating the reproducibility of psychological science, Science (2015) 349.
[8] J.A. Ioannidis, Contradicted and initially stronger effects in highly cited clinical research, JAMA 294 (2005) 218–228.
[9] J.P. Ioannidis, Failure to replicate: sound the alarm, Cerebrum (Nov–Dec, 2015) (cer-12a-5).
[10] National Institutes of Health Office of Intramural Research, Available from: https://grants.nih.gov/reproducibility/index.htm, .
[11] U.S. Government Accountability Office, Most Drugs Withdrawn in Recent Years Had Greater Health Risks for Women 2001, Available from: http://www.gao.gov/products/GAO-01-286R, .
[12] L.D. McCullough, Z. Zeng, K.K. Blizzard, I. Debchoudhury, P.D. Hurn, Ischemic nitric oxide and poly (ADP-ribose) polymerase-1 in cerebral ischemia: male toxicity, female protection, J. Cereb. Blood Flow Metab. 25 (2005) 502–512.
[13] W. Malorni, I. Campesi, E. Straface, S. Vella, F. Franconi, Redox features of the cell: a gender perspective, Antioxid. Redox Signal. 9 (2007) 1779–1801.
[14] E. Straface, L. Gambardella, M. Brandani, W. Malorni, Sex differences at cellular level: “cells have a sex”, Handb. Exp. Pharmacol. (2012) 49–65.
[15] C. Penaloza, B. Estevez, S. Orlanski, M. Sikorska, R. Walker, C. Smith, et al., Sex of the cell dictates its response: differential gene expression and sensitivity to cell death inducing stress in male and female cells, FASEB J. 23 (2009) 1869–1879.
[16] E. Ortona, P. Matarrese, W. Malorni, Taking into account the gender issue in cell death studies, Cell Death Dis. 5 (2014) e1121.
[17] N.R. Jog, R. Caricchio, Differential regulation of cell death programs in males and females by Poly (ADP-Ribose) Polymerase-1 and 17beta estradiol, Cell Death Dis. 4 (2013) e758.
[18] B. Manwani, K. Bentivegna, S.E. Benashski, V.R. Venna, Y. Xu, A.P. Arnold, et al., Sex differences in ischemic stroke sensitivity are influenced by gonadal hormones, not by sex chromosome complement, J. Cereb. Blood Flow Metab. 35 (2015) 221–229.
[19] U. Nussinovitch, Y. Shoenfeld, The role of gender and organ specific autoimmunity, Autoimmun. Rev. 11 (2012) A377–85.
[20] S. Bhinder, A. Singh, V. Majithia, Membranous (class V) renal disease in systemic lupus erythematosus may be more common than previously reported: results of a 6-year retrospective analysis, Am J Med Sci 339 (2010) 230–232.
[21] K.J. Berkley, Sex differences in pain, Behav. Brain Sci. 20 (1997) 371–380 (discussion 435-513).
[22] J.S. Mogil, B. A., Sex and gender differences in pain and analgesia, in: I. Savic (Ed.), Sex Differences in the Human Brain, Their Underpinnings and Implications, Elsevier, Amsterdam, 2010, pp. 141–158.
[23] B.E. Engstrom, F.A. Karlsson, L. Wide, Marked gender differences in ambulatory morning growth hormone values in young adults, Clin. Chem. 44 (1998) 1289–1295.
[24] S. Masuda, K. Ichihara, H. Yamanishi, Y. Hirano, Y. Tanaka, T. Kamisako, Evaluation of menstrual cycle-related changes in 85 clinical laboratory analytes, Ann. Clin. Biochem. 53 (2016) 365–376.
[25] P.J. Gunn, B. Middleton, S.K. Davies, V.L. Revell, D.J. Skene, Sex differences in the circadian profiles of melatonin and cortisol in plasma and urine matrices under constant routine conditions, Chronobiol. Int. 33 (2016) 39–50.
[26] I. Abou-Gareeb, S. Lewallen, K. Bassett, P. Courtright, Gender and blindness: a meta-analysis of population-based prevalence surveys, Ophthalmic Epidemiol. 8 (2001) 39–56.
[27] J.A. Clayton, A.F. Davis, Sex/gender disparities and women's eye health, Curr. Eye Res. 40 (2015) 102–109.
[28] M. Zetterberg, Age-related eye disease and gender, Maturitas 83 (2016) 19–26.
[29] Food and Drug Administration, FDA Drug Safety Communication: Risk of Next-morning Impairment After Use of Insomnia Drugs, FDA requires lower recommended doses for certain drugs containing zolpidem (Ambien, Ambien CR, Edluar, and Zolpimist). Available from: https://www.fda.gov/drugs/drugsafety/ucm334033.htm, .
[30] Food and Drug Administration, FDA Drug Safety Communication: FDA approves new label changes and dosing for zolpidem products and a recommendation to avoid driving the day after using Ambien CR 2013, Available from: https://www.fda.gov/Drugs/DrugSafety/ucm352085.htm, .
[31] Rabin, R. C. Group, The Drug-Dose Gender Gap, The New York Times, 2013, p. D4.
[32] P.M. Ridker, N.R. Cook, I.M. Lee, D. Gordon, J.M. Gaziano, J.E. Manson, et al., A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women, N. Engl. J. Med. 352 (2005) 1293–1304.
[33] J.S. Berger, M.C. Roncaglioni, F. Avanzini, I. Pangrazzi, G. Tognoni, D.L. Brown, Aspirin for the primary prevention of cardiovascular events in women and men: a sex-specific meta-analysis of randomized controlled trials, JAMA 295 (2006) 306–313.
[34] R. Shaw, M.F. Festing, I. Peers, L. Furlong, Use of factorial designs to optimize animal experiments and reduce animal use, ILAR J. 43 (2002) 223–232.
[35] L.M. Collins, J.J. Dziak, R. Li, Design of experiments with multiple independent variables: a resource management perspective on complete and reduced factorial designs, Psychol. Methods 14 (2009) 202–224.
[36] M.F. Festing, Randomized block experimental designs can increase the power and reproducibility of laboratory animal experiments, ILAR J. 55 (2014) 472–476.
[37] G.J. De Vries, E.F. Rissman, R.B. Simerly, L.Y. Yang, E.M. Scordalakes, C.J. Auger, et al., A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits, J. Neurosci. 22 (2002) 9005–9014.
[38] J.D. Greenspan, R.M. Craft, L. LeResche, L. Arendt-Nielsen, K.J. Berkley, R.B. Fillingim, et al., Studying sex and gender differences in pain and analgesia: a consensus report, Pain 132 (Suppl. 1) (2007) S26–45.
[39] S. Kerkhofs, S. Denayer, A. Haelens, F. Claessens, Androgen receptor knockout and knock-in mouse models, J. Mol. Endocrinol. 42 (2009) 11–17.
[40] V.R. Walker, K.S. Korach, Estrogen receptor knockout mice as a model for endocrine research, ILAR J. 45 (2004) 455–461.