

JANUARY 1 1999 VOL. 51 NO. 1
THGNBO 51(1) 1-448 (1999)
ISSN 0093-691X

Theriogenology.
51, no. 1 (Jan. 1 1999)
General Collection
1 TH677K
Received: 03-01-1999

THERIOGENOLOGY

AN INTERNATIONAL
JOURNAL OF
**ANIMAL
REPRODUCTION**

VICTOR M. SHILLE
Editor



PROPERTY OF THE
NATIONAL
LIBRARY OF
MEDICINE



Editorial Office: Manuscripts should be submitted to the Theriogenology Editorial Office, 2015 SW 16th Avenue, Box 100136, Health Sciences Center, University of Florida, Gainesville, FL 32610-0136, USA. Telephone: (352) 392-4700, ext. 5645. FAX: (352) 392-9652, E-mail: therio@mail.vetmed.ufl.edu

Editor: Victor M. Shille

Assistant Editor: Eve Cech

Theriogenology (ISSN 0093-691X) is published sixteen times (monthly in February, March, May, June, August, September, November, December; semi-monthly in January, April, July, and October) two volumes per year, by Elsevier Science Inc., 655 Avenue of the Americas, New York, NY 10010.

This journal is listed in Bibliography of Reproduction; *Biological Abstracts*; *Chemical Abstracts*; *Current Contents*; *FO:VM*™; *Science Citation Index*; *Index Medicus/MEDLINE*; *Derwent Veterinary Drug File*; and *Elsevier BIOBASE/Current Awareness in Biological Sciences*.

For Customers in Europe, The CIS and Japan: Institutional Rate NLG 1,576.00; Personal Rate NLG 493.00. For Customers in all other Countries: Institutional Rate US \$800.00; Personal Rate US \$250.00.

Any enquiry related to subscriptions should be sent to:

The Americas: Elsevier Science, Customer Support Department, PO Box 945, New York, NY 10010, USA [Tel: (+1)212-633-3730/1-888 4ES-INFO. Fax: (+1) 212-633-3680. E-mail: usinfo@elsevier.com].

Japan: Elsevier Science, Customer Support Department, 9-15 Higashi-Azabu 1-chome, Minato-ku, Tokyo 106, Japan [Tel: (+3) 5561-5033. Fax: (+3) 5561-5047. E-mail: kyf04035@niftyserve.or.jp].

Asia Pacific (excluding Japan): Elsevier Science (Singapore) Pte Ltd, No. 1 Temasek Ave., 17-01 Millenia Tower, Singapore 039192. [Tel: (+65) 434-3727. Fax: (+65) 337-2230. E-mail: asiainfo@elsevier.com.sg].

Rest of the World: Elsevier Science, Customer Service Department, PO Box 211, 1001 AE Amsterdam, The Netherlands. [Tel: (+31) 20-485-3757. Fax: (+31) 20-485-3432. E-mail: nlinfo@elsevier.nl].

Claims for missing issues can be honored only up to three months for domestic addresses, six months for foreign addresses. Duplicate copies will not be sent to replace one undelivered through failure to notify Elsevier of change of address. Single copy and back volume information available upon request.

POSTMASTER: Send address changes to *Theriogenology*, Elsevier Science Inc., 655 Avenue of the Americas, New York, NY 10010.

For Advertising Orders & Inquiries: *North & South America:* Tino DeCarlo, Elsevier Science Inc., 655 Avenue of the Americas, New York, NY 10010-5107, USA. Tel: (212) 633-3815; Fax: (212) 633-3820; E-Mail: tdecarlo@elsevier.com *International:* Advertising Department, Elsevier Science, The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, United Kingdom. Tel: (+44) (0) 1865 843565; Fax: (+44) (0) 1865 843976; E-Mail: media@elsevier.co.uk *Japan:* Advertising Department, Elsevier Science K.K., Tel: (81) (3) 5561 5033; Fax: (81) (3) 5561 5047.

No responsibility is assumed by the Publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use of operation of any methods, products, instructions or ideas contained in the material herein. Because of rapid advances in the medical sciences, the Publisher recommends that independent verification of diagnosis and drug dosages should be made.

© Elsevier Science Inc., 1998. All rights reserved. Periodical postage paid at New York, NY and at additional mailing offices. This journal has been registered with the Copyright Clearance Center Inc. Consent is given for copying of articles for personal or internal use of specific clients. This consent is given on the condition that the copier pay through the Center the per-copy fee stated in the cost on the first page of each article for copying beyond that permitted by the U.S. Copyright Law. If no code appears on an article, the author has not been given broad consent to copy and permission to copy must be obtained directly from the author. This consent does not extend to other kinds of copying; such as for general distribution, resale, advertising and promotional purposes, or for creating new collective works. All inquiries regarding copyright material from this publication, other than reproduction through the Copyright Clearance Center, should be directed to Elsevier Science Ltd., PO Box 800, Oxford OX5 1DX, U.K. Phone: 44-1865 843830; Fax: 44-1865 853333; E-mail: permissions@elsevier.co.uk

∞™ The paper meets the requirements of ANSI Standard Z39.48-1992 (Permanence of Paper).

INSEMINATION OF HEIFERS WITH SEXED FROZEN OR SEXED LIQUID SEMEN

G.E. Seidel, Jr.¹, D.G. Cran², L.A. Herickhoff², J.L. Schenk², S.P. Doyle³ and R.D. Green³

¹ Animal Reproduction and Biotechnology Laboratory, and ³Department of Animal Sciences, Colorado State University, Fort Collins, CO 80523

²XY, Inc., Fort Collins, CO 80525 USA

Angus heifers 13-15 mo of age in good body condition were synchronized by feeding MGA for 14 days followed by 25 mg of prostaglandin F-2-alpha 16 or 18 days later and inseminated 6-26 h after observed standing estrus. Freshly collected semen from four 14-26 mo old bulls was incubated in 224 µM Hoechst 33342 at 400 x 10⁶ sperm/ml in a TALP medium for 1 h at 34°C and then diluted to 100 x 10⁶ sperm/ml for sorting. Sperm were sorted by sex chromosomes on the basis of DNA content. An argon laser, emitting 150 mW at 351 and 364 nm, was used on a MoFlo® flow cytometer/cell sorter modified for sperm sorting operating at 50 psi with 2.9% Na citrate as sheath fluid. X chromosome-bearing sperm (~90% purity as verified by reanalyzing sonicated sperm aliquots for DNA) were collected at ~900 live sperm/sec into 14-ml tubes containing 2 ml egg yolk extender. Collected sperm were centrifuged and suspended to 10⁷ live sperm/ml in Cornell Universal Extender for liquid semen or 20% egg yolk in 2.9% Na citrate for frozen semen. Sexed semen to be frozen was cooled to 5°C over 90 min and loaded into 0.25-ml straws. After glycerolization straws were frozen by standard procedures. Straws of liquid semen were transported at 18°C in a temperature-controlled incubator 240 km for insemination 5 to 9 h after sorting. Sexed semen was inseminated using side-opening blue sheaths (IMV), one half of each straw into each uterine horn. As a standard control, semen from the same bulls had been frozen in 0.5-cc straws by standard procedures, thawed at 37°C for 30 sec, and inseminated into the uterine body. Treatments were balanced over the 4 bulls and 2 inseminators. Pregnancy was determined ultrasonically 62-65 days after insemination when fetuses also were sexed "blindly" (see table).

Treatment	No. heifers bred	No. sperm/dose	No. pregnant	No. ♀ fetuses
Sexed liquid	37	5 x 10 ⁵	11 (30%) ^a	11 (100%) ^a
Sexed frozen	35	1 x 10 ⁶	18 (51%) ^{a,b}	17 (94%) ^a
Frozen control	37	40 x 10 ⁶	27 (73%) ^b	16 (59%) ^b

^{a,b} Values without common superscripts differ (P<0.02).

Pregnancy rates with liquid and frozen sexed semen were 41 and 70% of the control; this difference was statistically significant (P<0.05) for liquid sexed semen. The frozen semen control had 40 to 80 X more sperm than the sexed semen treatments. 28 of 29 fetuses (97%) were female when sexed semen was used.