



Industria y Comercio
SUPERINTENDENCIA

PCT

SOLICITUD

PATENTE DE INVENCION

21. EXPEDIENTE N°

03-54825

24. TÍTULO

**DISPOSICION PARA FERTILIZACION IN-VITRO CON ESPERMATOSIS SEPARADOS ENTRE
DIFERENTES POBLACIONES QUE CONTIENEN CROMOSOMAS X Y CROMOSOMAS Y.**

25. CLASIFICACIÓN INTERNACIONAL

A 01 N¹/00; AG K 35/52.

26. SOLICITANTE

EL, INC. Y COLORADO STATE UNIVERSITY

DOMICILIO

FORT COLLINS, Colorado, Estado, Unidos de America

27. APODERADO

Emilio Ferrero.

28. BOGOTÁ, D. C.



Industria y Comercio
SUPERINTENDENCIA

PETITORIO



03 054825 00

SUPERINDUSTRIA Y COMERCIO

Radicacion : 03054825 00000000 Folios: 123 n
Fecha (ORD): 2003-06-27 17:50:59
Trámite : 011 PCT-PATENT 379 FASE INCI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

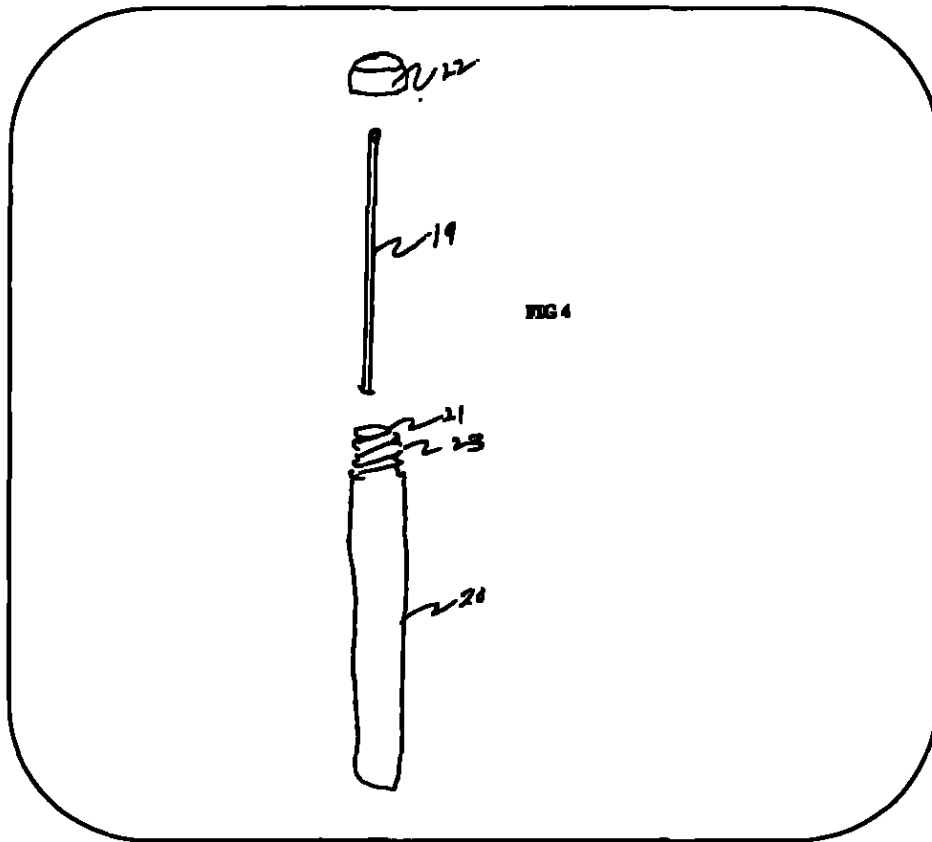
**FORMULARIO UNICO DE SOLICITUD DE PATENTE
PCT
ENTRADA EN FASE NACIONAL**

1 SOLICITUD DE: ☒ Patente de Invención. ☐ Patente de Modelo de Utilidad.		
☐ Capítulo I. ☒ Capítulo II.		
2 SOLICITANTE (71)	Nombre: XY, INC. Y COLORADO STATE UNIVERSITY sociedades constituidas y existentes bajo las leyes del estado de Colorado, Estados unidos de América Dirección: 1108 North Lemay Avenue Fort Collins Colorado- 80524, Estados Unidos de América Colorado State University Research Foundation, Room 410, 610 South Howes Street, Fort Collins, Colorado 80522, Estados Unidos de América Domicilio: Fort Collins, Colorado, Estados Unidos de América,	IDENTIFICACION C.C. ☐ NIT ☐ C.E. ☐ Otro ☐ Cual _____ Número: _____
3 REPRESENTANTE O APODERADO	Nombre: EMILIO FERRERO Dirección: Carrera 4ª. No. 72-35 Teléfono: 347 36 11 Fax:: 217 92 11 E-mail: clientes@camyp.com. Domicilio: Bogotá, Colombia.	IDENTIFICACION C.C. ☒ NIT ☐ C.E. ☐ Otro ☐ Cual _____ Número: 438.019 T.P. 31.929
4 INVENTOR (ES) (72)	1- George, E. SEIDEL 2- Kwang Tae SUH 3- Kehuan LU 1,2,3 US-1108 North Lemay Avenue, Fort Collins Colorado 80524, Estados Unidos de América	
5 PCT (86) Solicitud Internacional No.: PCT/US01/45237 Publicación Internacional No. WO 02/43486 A1 Fecha: 29 de noviembre de 2001 Fecha : 6 de junio de 2002		

6	Título (54) <u>DISPOSICION PARA FERTILIZACION IN-VITRO CON ESPERMATOZOIDES SEPARADOS DENTRO DE POBLACIONES QUE CONTIENEN CROMOSOMA X Y CROMOSOMA Y</u>			
7	Clasificación Internacional (51) <u>A01N 1/00, AGK 35/52.</u>			
8	Prioridad SI ☒ NO ☐	(33) País de Origen <u>US</u> <u>US</u>	(32) Fecha <u>29 de noviembre de 2000</u> <u>29 de noviembre de 2000</u>	(31) Número de Solicitud <u>60/253.785</u> <u>60/253.787</u>
9	Comprobante de pago No.: <u>03-44.015</u> Fecha: <u>24 de junio de 2003</u>			
10	Anexos:			
☒	Comprobante de pago de la tasa de presentación de la solicitud por \$ 400.000			
☐	Comprobante de pago por reivindicación de prioridad.			
☒	Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.			
☒	Poderes, si fuere el caso.			
☐	Documento de cesión del inventor al solicitante o a su causante.			
☐	Declaraciones.			
☒	Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos)			
☒	Traducción de la solicitud Internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos)			
☒	Copia del examen preliminar efectuado por la IPEA.			
☐	Traducción del examen preliminar efectuado por la IPEA.			
☐	De ser el caso, copia del contrato de acceso.			
☐	De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.			
☐	De ser el caso, certificado de depósito del material biológico.			
☒	Arte final 12x12			
☐	De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.			
☐	Otros			

11

FIGURA CARACTERISTICA:



12

Solicito la concesión de la patente,

NOMBRE: EMILIO FERREROFIRMA: *Emilio Ferrero*
C.C. 438.019 de Usaquén T.P. 31.929

MRE/mrm-ld-

13534
41012



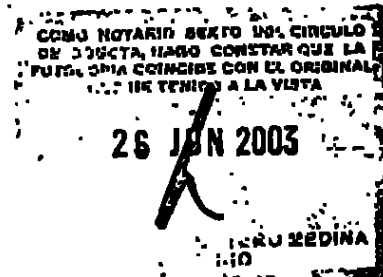
APOSTILLE
(Convention de La Haye du 5 October 1961)

1. Country: United States of America
This public document
2. has been signed by CHERYL A SWANSON
3. acting in the capacity of Notary Public, State of Colorado
4. bears the seal/stamp of the CHERYL A SWANSON, Notary Public, State of Colorado

CERTIFIED

5. at Denver, Colorado
6. this 6th day of May, 2003
7. By DONETTA DAVIDSON, Secretary of State, State of Colorado
8. Certificate Number 67398.
9. (OFFICIAL SEAL)
10. Signature:

Donetta Davidson



Representación y Poder con autenticación notarial y Apostille para patentes, modelos de utilidad, diseños industriales, marcas de fábrica, nombres comerciales, enseñas, nombres de dominio, lemas comerciales, licencias.

Representation and Power-of-Attorney with notarial authentication and Apostille for patents, utility models, industrial designs, trademarks, commercial names, trade emblems, domain names, slogans, licenses.

REPRESENTACION Y PODER

REPRESENTATION AND POWER-OF-ATTORNEY

Yo (nosotros), abajo firmado (s)/I (we), the undersigned

Mervyn Jacobson

domiciliado (s) en/of XY, Inc. Fort Collins, Colorado 80524, Estados Unidos

hereby ratify the patent applications No. 02.101.971 and No. 02.112.918 filed by EMILIO FERRERO on November 8, 2002, and December 12, 2002 respectively, and

por el presente nombramos a EMILIO FERRERO, tpa. 31929; GONZALO PERDOMO, tpa. 831; LUZ CLEMENCIA DE PAEZ, tpa. 23555 y GERMAN CAVELIER, tpa. 832, domiciliados en la Carrera 4a N° 72-35, Bogotá, Colombia, en obediencia a lo dispuesto en el párrafo 1o del Artículo 543 del Código de Comercio, el primero como principal y los demás como suplentes, en su orden, como mis (nuestros) representantes en todos los asuntos de propiedad industrial con facultad para recibir notificaciones y nombrar apoderados judiciales o extrajudiciales. El primer representante suplente reemplazará al principal en caso de ausencia o impedimento temporal o definitivo de aquél. La misma regla se aplicará cuando el segundo representante suplente haya de reemplazar al primero, o el tercero al segundo. En tales casos, el representante principal, o el primero, o segundo suplente, en su caso, o ambos, declararán su intención de no ejercer la representación mediante declaración autenticada por Notario Público en Colombia, o por Notario u otro Funcionario Público, o Cónsul de Colombia, en el extranjero; y si se ratifica de impedimento, con el certificado respectivo. Si alguno de los nombrados faltare definitivamente, el siguiente tomará su lugar. Cuando cesare la ausencia o impedimento del representante principal, o de su primero o segundo suplentes, en su caso, podrán ejercer la representación, en su orden, uno u otros según el caso, asumiéndola por solo hecho de ejercerla, cesando en ese momento el ejercicio de la representación por el primero, segundo o tercer suplente en su caso.

Además, confiero (conferimos) poder a los abogados arriba nombrados, para obtener la protección de mi (nuestra) propiedad industrial y contra la competencia desleal, a cuyo efecto autorizo (amos) a los dichos apoderados para que me (nos) representen ante cualesquiera entidades o personas, ejerzan o no jurisdicción, especialmente ante las del orden administrativo, contencioso-administrativo y judicial, así como los tribunales de arbitramento, en todo lo concerniente a los siguientes asuntos: a) Obtención de patentes de invención, modelos de utilidad y diseños industriales; el registro de marcas de fábrica, colectivas, defensivas, de servicio, lemas comerciales, nombres comerciales,

enseñas, nombres de dominio, variedades vegetales y denominaciones de origen; su renovación, traspaso, cambio de nombre o domicilio, cancelación voluntaria; y el registro de licencias de uso de esos derechos; b) Las acciones de competencia desleal, protección del consumidor, oposiciones u observaciones, cancelación administrativa y judicial, nulidad, medidas cautelares, concesión de licencias, la tutela, la protección de secretos industriales, y en general los juicios civiles, penales, administrativos o contencioso-administrativos relacionados con estos derechos; acciones y procesos que promovamos o se nos

in due compliance with the terms of paragraph 1st. of article 543 of the Commercial Code, do hereby appoint EMILIO FERRERO, tpa. 31929; GONZALO PERDOMO, tpa. 831; LUZ CLEMENCIA DE PAEZ, tpa. 23555 and GERMAN CAVELIER, tpa. 832, domiciled at Carrera 4a N° 72-35, Bogotá, Colombia, the first as principal and the others as alternates, in the order of their appointment, as our representatives for all industrial property matters with faculty to receive notifications and to appoint attorneys in and out of Court. The first alternate shall replace the principal in case of his absence or of temporal or definitive impediment. The same rule will apply when the second alternate representative is to replace the first one, or when the third is to replace the first or second alternates in their turn, or both of them, second. In such cases, the principal representative, or the first or second alternates in their turn, or both of them, shall state their intention not to act as representatives through a declaration given before a Notary Public in Colombia, or before a Notary or another Public Official or a Colombian Consul abroad; and in case of impediment, the appropriate certificate shall suffice. If any of the appointees were to be definitely absent, the following alternate shall take his place. When the absence or impediment of the principal representative, or of the first or second alternate in their turn ceases, the representation can be taken up again in the sequence by the principal, the first or second alternate depending on case, and the third or its exercise will suffice. The exercise of the representation by the first, second or third alternates in their turn shall end each time. In addition, we grant a Power of Attorney in favor of the above-named attorneys-at-law so that they may obtain the protection of my (our) industrial property and against the unfair competition; for which purpose I (we) authorize the said attorneys to represent me (us) before any authorities or persons, with or without jurisdiction, specially those administrative, administrative contentious and judicial, as well as before Arbitration Court, in all matters concerning the following: a) Obtainment of patents, utility models and industrial designs; the registration of trademarks, collective, defensive and service marks,

slogans, commercial names, trade emblems, domain names, vegetal varieties and denominations of origin; its renewal, assignment, change of name or domicile, voluntary cancellation; and the registration of licenses of use of the above rights; b) Actions of unfair competition, protection to the consumer, oppositions or observations, administrative or Court cancellation, nullity, infringement, granting of licenses, the action for writ mandamus, the protection of trade secrets, and in general the civil, penal, and administrative suits relating to those rights; actions and suits instituted by me (us) or against me (us). And that in all the above matters

prueben. Y para que en todos los asuntos arriba determinados, puedan sustituir este mandato, transigir, conciliar, admitir los hechos del proceso, desistir, cancelar, recibir, renunciar, y ratificar los actos de agentes oficiosos.

they may substitute this Power of Attorney, compromise, conciliate, admit the facts of the case, desist, cancel, receive, resign, and ratify acts done by representatives without a power of attorney.

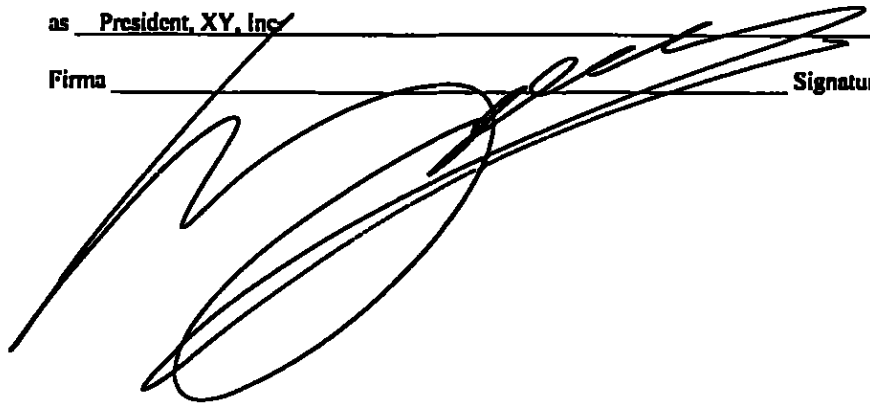
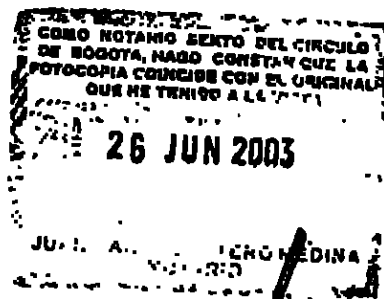
Dado y firmado hoy/Given and signed this 3rd day of FEB, 2003

en/in Fort Collins, Colorado

por/by Mervyn Jacobson

as President, XY, Inc

Firma _____ Signature

CERTIFICADO NOTARIAL (INDIVIDUO)

A los 3 días del mes de Febrero
de 2003 ante mí, Notario Público o Funcionario Autorizado
de l Estado de Colorado EEUU
compareció(eron) personalmente _____

a quien(es) doy fe de conocer y me consta que es(son) la(s)
persona(s) firmante(s) del documento que precede, y quien(es)
tiene(n) capacidad legal para otorgarlo

Notario Público o Funcionario Autorizado -- Notary Public or Attesting Official

CERTIFICADO NOTARIAL (SOCIEDAD)

Hoy 3 de FEBRERO de 2003
Compareció(eron) ante mí
Mervyn Jacobson
quien(es) conozco personalmente y que firma(n) este
documento en representación de la Sociedad/compañía
denominada XY, Inc.

Certifico:

1. Que he visto los siguientes documentos:*

Informe de la Sociedad or Compañía de
Responsabilidad Limitada fechado 20 Junio,
1998, presentado con el Secretario de Estado
del Estado de Colorado, lo cual es una copia
fiel y correcta obtenida de www.sos.state.co.us
y leído por el suscrito

2. Que dichos documentos prueban lo siguiente:

2.1. Que XY, Inc.

es una Sociedad/Compañía legalmente constituida y existente
de acuerdo con las leyes de l Estado de Colorado,
Estados Unidos de America

2.2. Que el domicilio de dicha Sociedad/Compañía es
1108 North Lemay Avenue, Fort Collins,
Colorado 80524

2.3. Que el objeto u objetos para los cuales se otorga el presente
poder están comprendidos dentro del objeto social de la
mencionada Sociedad/Compañía.

2.4. Que el(los) Sr.(Sres.) Mervyn Jacobson, Presidente
de XY, Inc.
está(n) autorizado(s) para otorgar el presente Poder.

Ciudad Fort Collins

Estado Colorado, Estados Unidos de America

Hoy 3 Febrero de 2003

Notario Público o Funcionario Autorizado -- Notary Public or Attesting Official

* Los documentos deben identificarse con fecha y origen

NOTARIAL CERTIFICATE (INDIVIDUAL)

On this 3 day of February
2003 before me, a Notary Public or Attesting Official of
The State of Colorado USA
personally appeared Mervyn Jacobson
to me known, and known to me to be the person(s) who
executed that foregoing document, and who (has) legal
capacity to execute it.

My Commission Expires 08-21-2003

NOTARIAL CERTIFICATE (CORPORATION)

My Commission Expires 08-21-2003

On this 3 day of February 2003
appeared before me
Mervyn Jacobson
who(m) I know personally and who(m) signed this document as
representative(s) of the Corporation/Company named:
XY, Inc.

I certify:

1. That I have seen the following documents:*

Biennial Report of Corporation or Limited Liability
Company, dated June 20, 1998, and filed with the
Colorado Secretary of States office, a true and correct
copy of which was downloaded from
www.sos.state.co.us and read by the undersigned.

2. That the above documents duly prove the following:

2.1. That XY, Inc.

is a Corporation/Company legally constituted and in existence
in accordance with the laws of the State of Colorado, United
States of America.

2.2. That the domicile of said Corporation/Company is

1108 North Lemay Avenue, Fort Collins, Colorado 80524

2.3. That the purpose or purposes for which the present Power
of Attorney is granted are comprised within the corporation
purposes set forth in the Articles of Incorporation of the mentioned
Corporation/Company.

2.4. That Mr.(Messrs.) Mervyn Jacobson, President of XY,
Inc. is(are) authorized to grant the present Power of Attorney.

City: Fort Collins

State: Colorado, United States of America

This 3 day of February 2003

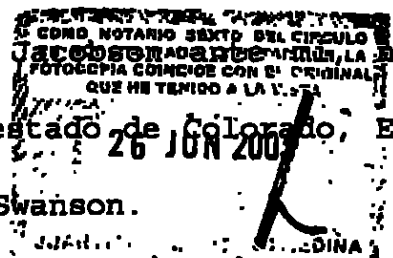
Craig R. Miles
Santangelo Law Offices, P.C.

My commission Expires 8/21/2003

* Documents must be identified with date and origin

TRADUCCIÓN OFICIAL NÚMERO 071/2003 REALIZADA POR EDUARDO CALADO NOGUERA, TRADUCTOR OFICIAL APROBADO POR RESOLUCIÓN 541 DEL 27 DE FEBRERO DE 1976 Y DEBIDAMENTE INSCRITO ANTE EL MINISTERIO DE RELACIONES EXTERIORES DE COLOMBIA, DE LOS SELLOS Y AUTENTICACIONES QUE APARECEN EN EL DOCUMENTO DE REPRESENTACIÓN, RATIFICACIÓN Y PODER OTORGADO POR MERVYN JOHNSON A NOMBRE DE XY INC, DOMICILIADA EN FORT COLLINS, COLORADO 80524, ESTADOS UNIDOS DE AMÉRICA, POR EL CUAL RATIFICAN LAS SOLICITUDES DE PATENTE NÚMEROS 02.101.971 Y 02.112.918 , PRESENTADAS POR EL DOCTOR EMILIO FERRERO LOS DIAS 8 DE NOVIEMBRE DE 2002 Y 12 DE DICIEMBRE DE 2002, RESPECTIVAMENTE Y QUE IGUALMENTE OTORGA PODER A LOS DOCTORES EMILIO FERRERO, TPA 31929, GONZALO PERDOMO, TPA 831, LUZ CLEMENCIA DE PAEZ, TPA 23555 Y GERMÁN CAVELIER, TPA 832. Dado y firmado hoy 3 de febrero de 2003 en Fort Collins, Colorado, Mervyn Jacobson como presidente de XY, Inc. Firmado: ilegible.

Certificado notarial de fecha 3 de febrero de 2003 por el cual se certifica la comparecencia de Mervyn Jacobson como representante de XY, Inc., la existencia de la empresa y su domicilio y la capacidad de dicha persona para otorgar el presente poder. Ciudad : Fort Collins, Colorado, Estados Unidos de América. Firmado: Cheryl A. Swanson.



Sello: Cheryl A. Swanson, notario público, estado de Colorado.

Certificado notarial en inglés y en español, de fecha 3 de febrero de 2003 para certificar la comparecencia de Mervyn Jacobson como representante de XY, Inc., la existencia de la empresa y su domicilio y la capacidad de dicha persona para otorgar el presente poder. Ciudad : Fort Collins, Colorado, Estados Unidos de America, 3 de febrero de 2003. Firmado: Craig R. Miles, Santangelo Law Offices, P.C.

Firmado: Cheryl A. Swanson, 3 de febrero de 2003.

Sello: Estado de Colorado, 1876

APOSTILLA:

(Convención de La Haya del 5 de octubre de 1961)

1. Estados Unidos de América

Este documento público

2. ha sido firmado por CHERYL A. SWANSON

3. quien actúa en su calidad de NOTARIO PÚBLICO, ESTADO DE
COLORADO

4. lleva el sello de CHERYL A. SWANSON, NOTARIO PÚBLICO,
ESTADO DE COLORADO

CERTIFICADO

5. en Denver, Colorado

6. el 6 de mayo de 2003

7. por DONETTA DAVIDSON, Secretario de estado, estado de
Colorado.

8. Número 67398

9. Sello oficial

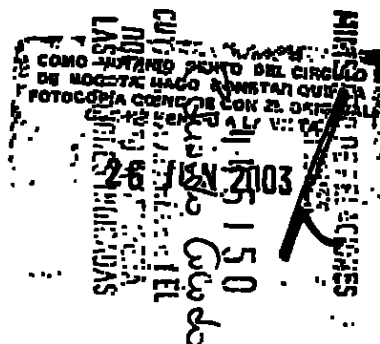
10. Firma: DONETTA DAVIDSON

COLO-13534-841-001-4

WPCL002G ID 4468

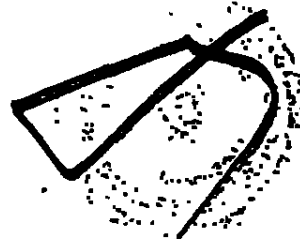
Bogotá, 20 de mayo de 2003.

Eduardo Calado
Eduardo Calado Negrete
TRADUCTOR OFICIAL
INGLÉS - ESPAÑOL
Res 541, febrero de 1997



NO SE ASUME
RESPONSABILIDAD
POR EL CONTENIDO
DE ESTE DOCUMENTO
2003 MAY 21 12:13
NOTARY PUBLIC
STATE OF COLORADO

CO. ... LE ... TAFE ...
...
Roberto Aguero
...
S. ...
...



...
COMO NOTARIO SEITO DEL CIRCULO
DE BOGOTA HAGO CONSTAR QUE LA
FOTOCOPIA COINCIDE CON EL ORIGINAL
QUE ME FUE COPIADA
26 JUN 2003
JUAN MANUEL SOTERO MEDINA
NOTARIO

(19) World Intellectual Property Organization
International Bureau



INTERNATIONAL BUREAU OF PATENT COOPERATION
358, rue de la Confiance
CH-1202, Genève, Suisse
P.O. Box 6809
CH-1211, Genève, Suisse

(43) International Publication Date
6 June 2002 (06.06.2002)

PCT

(10) International Publication Number
WO 02/43486 A1

- (51) International Patent Classification: A01N 1/00 Room 410, 601 South Howes Street, Fort Collins, CO 80522 (US).
- (21) International Application Number: PCT/US01/45237 /
- (22) International Filing Date: 29 November 2001 (29.11.2001)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/253,785 29 November 2000 (29.11.2000) US
60/253,787 29 November 2000 (29.11.2000) US
- (71) Applicants (for all designated States except US): XY, INC. (US/US); 1108 North Lemay Avenue, Fort Collins, CO 80524 (US). COLORADO STATE UNIVERSITY (US/US); Colorado State University Research Foundation.
- (72) Inventors: and /
- (73) Inventors/Applicants (for US only): SEIDEL, George, E. (US/US); c/o XY, Inc., 1108 North Lemay Avenue, Fort Collins, CO 80524 (US). SUH, Tae, Kwang (US/US); c/o XY, Inc., 1108 North Lemay Avenue, Fort Collins, CO 80524 (US). LU, Kehuan (US/US); c/o XY, Inc., 1108 North Lemay Avenue, Fort Collins, CO 80524 (US).
- (74) Agent: MILES, Craig, R.; Santangelo Law Offices, P.C., Third Floor, 125 South Howes, Fort Collins, CO 80521 (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EC, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE,

[Continued on next page]



WO 02/43486 A1

(54) Title: SYSTEM FOR IN-VITRO FERTILIZATION WITH SPERMATOZOA SEPARATED INTO X-CHROMOSOME AND Y-CHROMOSOME BEARING POPULATIONS

(57) Abstract: An IVF system for successfully utilizing spermatozoa separated into X-chromosome bearing and into Y-chromosome bearing population for insemination. The IVF system includes fertilization medium that can shorten the time from insemination to cleavage and a portable incubator for the transportation of maturing oocytes and inseminated oocytes comprising a straw (19) and an incubation element (20) that can be sealed with a cap (22).





GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv)) for US only

Published:

— with international search report

— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

SYSTEM FOR IN-VITRO FERTILIZATION WITH SPERMATOOZOA SEPARATED INTO X-CHROMOSOME AND Y-CHROMOSOME BEARING POPULATIONS

This application claims the benefit of United States Provisional Patent Application No. 60/253,787, filed November 29, 2000 and United States Provisional Patent Application No. 60/253,785, filed November 29, 2000, each hereby incorporated by reference herein.

I. TECHNICAL FIELD

Devices, compositions, and methods that improve the quality of embryos generated using in-vitro fertilization (IVF) with spermatozoa separated into X-chromosome bearing and Y-chromosome bearing populations.

II. BACKGROUND

An attractive feature of IVF is that many fewer spermatozoa can be required for insemination than for artificial insemination. However, IVF using spermatozoa separated into X-chromosome bearing and Y-chromosome bearing populations (separated spermatozoa) can necessitate modifications to conventional IVF techniques. This may be due in part to the pre-capacitation of such spermatozoa.

In most cases, the percentages of oocytes (oocyte, ootid, or ova, or plurality of same as appropriate to the application) fertilized with separated and unseparated spermatozoa are similar, and events during the first cell cycle are timed similarly for separated and unseparated spermatozoa. However, with conventional procedures, blastocyst production with separated spermatozoa can be 70%-90% of controls with spermatozoa that have not been separated. For example, development to blastocysts has been shown to be 17% with bovine oocytes inseminated with separated spermatozoa, compared with >25% which might be expected with IVF using unseparated spermatozoa as described in the journal article entitled "In Vitro Fertilization With Flow-Cytometrically-Sorted Bovine Sperm" Theriogenology 52: 1393-1405 (1999), hereby incorporated by reference.

Several factors may contribute to these results. One factor may be that staining of sperm with Hoechst 33342 appears to cause a decline in motility of spermatozoa. Another factor, may be the physical forces the spermatozoa are subject to during the separation process. As but one example, in flow cytometric separation of spermatozoa, spermatozoa exit the flow cytometer at nearly 100 km/h before impacting on the surface of the collection medium. During transit through the flow cytometer spermatozoa can be subjected to laser light at an intensity of over 100mW. While the transit time may only be 1-2μsec, this may affect the spermatozoal DNA, and thus, also effect subsequent embryonic development. The process of separating sperm with flow cytometry can also result in a highly diluted sample, 600,000 spermatozoa/mL or less, and subsequent centrifugation steps are necessary to provide concentrated spermatozoa suitable for insemination.

Another problem with utilizing separated spermatozoa in IVF techniques may be that the facility in which the spermatozoa are separated may be in a different location than where the male mammal from which the spermatozoa are collected is located, which may be different from where the female mammal from which the oocytes are collected is located, which may be a different location from where the in-vitro fertilization is to occur, and which may be a different location from where the female mammal into which the in-vitro cultured embryos are to be transferred. Conventionally, separated sperm may be cryopreserved and transported frozen to the facility at which the IVF techniques are administered. Maturing oocytes are conventionally transported to the facility at which the IVF techniques are administered in portable incubation systems. The maturing oocytes are then inseminated with previously frozen-thawed sperm cells. To avoid cryopreservation of sperm cells or as a convenience to the various facilities involved it may be beneficial to transport maturing oocytes directly to the facility separating the spermatozoa so that separated sperm cells can be added to the oocytes without cryopreservation. However, conventional IVF and in vitro culture of the resulting zygotes typically comprises a separate set of apparatus and procedures making it inconvenient, difficult, or impossible to inseminate and culture oocytes in the same facility in which spermatozoa are separated.

Even though X-chromosome bearing spermatozoa and Y-chromosome bearing spermatozoa have been differentiated by and separated based upon the difference in emitted fluorescence for many years, and even though separated spermatozoa have been used for some time with IVF techniques, and even though there is large commercial market for embryos produced with IVF techniques and separated spermatozoa, the above-mentioned problems have yet to be resolved.

As to the problems with conventional techniques of IVF using separated spermatozoa, and specifically separated spermatozoa, stained spermatozoa, or spermatozoa that are from previously frozen sperm, and with conventional strategies involving the transportation of separated sperm and maturing oocytes, the invention addresses each in a practical manner.

III. DISCLOSURE OF THE INVENTION

Accordingly, one of the broad objects of particular embodiments of the invention can be to provide devices, compositions and methods that provide transportation of inseminated oocytes, promotes cleavage of fertilized oocytes and improves the quality of embryos generated with techniques utilizing spermatozoa separated into X-chromosome bearing and Y-chromosome bearing populations.

Another broad object of particular embodiments of the invention can be to provide devices, compositions, and methods that promote cleavage and improve quality of embryos generated using IVF with spermatozoa that are derived from previously frozen sperm.

Another broad object of particular embodiments of the invention can be to provide devices, compositions, and methods that promote cleavage and improve quality of embryos generated using IVF with spermatozoa that have previously been stained with a DNA binding fluorochrome.

Another broad object of particular embodiments of the invention can be to provide medium for embryonic culturing that can contain non-essential amino acids.

Another broad object of the invention can be to provide apparatus and methods for transporting maturing oocytes and fertilized oocytes for the convenience of the end user(s) or to avoid cryopreservation of the spermatozoa used to fertilize oocytes.

Naturally further objects of the invention are disclosed throughout other areas of specification.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows an embodiment of the invention in which spermatozoa from fresh or previously frozen-thawed sperm are stained.

Figure 2 shows an embodiment of the invention for separating stained spermatozoa in to X-chromosome bearing and Y-chromosome bearing populations.

Figure 3 shows another view of an embodiment of the invention for separating stained spermatozoa in to X-chromosome bearing and Y-chromosome bearing populations.

Figure 4 shows an embodiment of a portable incubation system in which oocytes can be fertilized.

V. MODE(S) FOR CARRYING OUT THE INVENTION

The invention involves devices, methods, and compositions for the in-vitro insemination and fertilization of oocytes (oocyte, ootid, or ova, or plurality of same as appropriate to the application) and the culture of embryos resulting from such techniques.

Embodiments of the invention can include fresh spermatozoa, or spermatozoa from frozen-thawed sperm of numerous species of mammals. The invention should be understood not to be limited to the species of mammals cited by the specific examples within this patent application. Embodiments of the invention, for example, may include fresh spermatozoa or spermatozoa from frozen-thawed sperm of animals having

commercial value for meat or dairy production such as swine, bovids, ovids, equids, buffalo, or the like (naturally the mammals used for meat or dairy production may vary from culture to culture). It may also include fresh spermatozoa or spermatozoa from frozen-thawed sperm from individuals having rare or uncommon attribute(s), such as morphological characteristics including weight, size, or conformation, or other desired characteristics such as speed, agility, intellect, or the like. It may include frozen-thawed sperm from deceased donors, or fresh or frozen-thawed spermatozoa from rare or exotic mammals, such as zoological specimens or endangered species. Embodiments of the invention may also include fresh or frozen-thawed spermatozoa collected from primates, including but not limited to, humans, chimpanzees, gorillas, or the like, and may also include fresh or frozen-thawed spermatozoa from marine mammals, such as whales or porpoises.

Now referring primarily to Figure 1, in some embodiments of the invention, Hoechst 33342 stain (1) can be added to bovine spermatozoa contained in frozen-thawed sperm (2) to establish a concentration of 224 μ M. The incubation time of the spermatozoa contained in the frozen-thawed sperm (2) with the stain (1) can be about 190 minutes. In another embodiment of the invention, the stain (1) can be added to the bovine sperm (2) to establish a concentration of 2240 μ M and then incubated for about 60 minutes. Frozen-thawed sperm treated in either manner can improve the resolution of X-chromosome bearing from Y-chromosome bearing spermatozoa. Understandably, from application to application (such as frozen-thawed sperm from different species) the amount of incubation time and the specific concentration of stain can be adjusted to optimize the resolution of the X-chromosome bearing from Y-chromosome bearing spermatozoa.

With respect to the cleavage rates of inseminated oocyte(s), the increase in stain concentration up to at least 10X does not appear to have a depressive effect on either cleavage or embryonic development. Higher stain concentrations may actually be beneficial with respect to certain applications because the length of incubation time may be decreased improving percent cleavage. From application to application length of incubation time can be adjusted to optimize cleavage results or embryonic development, as desired.

Now referring primarily to Figures 2 and 3, a flow cytometer embodiment of the invention is shown which includes a sperm cell source (3) which acts to establish or supply stained spermatozoa or other type of stained cells to be analyzed by the flow cytometer. The sperm cells are deposited within a nozzle (4) in a manner such that the cells are surrounded by a sheath fluid (5). The sheath fluid (5) is usually supplied by some sheath fluid source (6) so that as the cell source (3) supplies sperm cells, the sheath fluid (5) is concurrently fed through the nozzle (4). In this manner it can be easily understood how the sheath fluid (5) forms a sheath fluid environment for the cells. Since the various fluids are provided to the flow cytometer at some pressure, they flow out of the nozzle (4) and exit at the nozzle orifice (7). By providing some type of oscillator (8) which may be very precisely controlled through an oscillator control (9), pressure waves may be established within the nozzle (4) and transmitted to the fluids exiting the nozzle (4) at nozzle orifice (7). Since the oscillator (9) thus acts upon the sheath fluid (5), the stream (10) exiting the nozzle orifice (7) eventually and regularly forms drops (11). Because the sperm cells are surrounded by a sheath fluid environment, the drops (11) may contain within them individually isolated (generally) cells or other items.

Since the drops (11) generally contain isolated sperm cells, the flow cytometer can distinguish and separate droplets based upon whether or not the appropriate sperm cell is contained within the drop. This is accomplished through a cell sensing system (12). The cell sensing system involves at least some type of sensor (13) which responds to the cells contained within each drop (11) as described by U.S. Patent No. 5135759, hereby incorporated by reference. As the Johnson patent explains for spermatozoa or sperm cells, although the staining and separation inventions can be understood to be used with a variety of frozen-thawed cells, the cell sensing system (13) may cause an action depending upon the relative presence or relative absence of the bound fluorochrome which may be excited by some stimulant such as the laser exciter (14). While each type of sperm cell can be stained by the stain or fluorochrome, as described above, the differing length of the X-chromosome and the Y-chromosome causes different amounts of stain to be bound. Thus, by sensing the degree of fluorescence emitted by the fluorochrome upon excitation it is possible to discriminate between X-bearing spermatozoa and Y-bearing spermatozoa by their differing fluorescence emission levels.

In order to achieve separation and isolation of the appropriate sperm cells, the signals received by sensor (14) are fed to some type of sorter discrimination system (15) which very rapidly makes a differentiation decision and can differentially charge each drop (11) based upon whether it has decided that the desired sperm cell does or does not exist within that drop (11). In this manner the separation or discrimination system (15) acts to permit the electrostatic deflection plates (16) to deflect drops (11) based on whether or not they contain the appropriate sperm cell. As a result, the flow cytometer acts to sort the sperm cells by causing them to land in one or more collectors (17). Thus by sensing some property of the sperm cells the flow cytometer can discriminate between sperm cells based on a particular characteristic and place them in the appropriate collector (17). In the system presently used to sort spermatozoa, the X-bearing spermatozoa droplets are charged positively and thus deflect in one direction, the Y-bearing spermatozoa droplets are charged negatively and thus deflect the other way, and the wasted stream (that is unsortable cells) is uncharged and thus is collected in an undeflected stream into a suction tube or the like.

Now referring primarily to Figure 3, the process can be even further understood. As shown in that figure, the nozzle (4) emits a stream (10) which because of the oscillator (8) (not shown in Figure 3) forms drops (11). Since the cell source (3) (not shown in Figure 3) may supply sperm cells (1) which have been stained according the invention, the magnitude of the fluorescent emission stimulated by the laser exciter (13) is differentially determined by sensor (14) so that the existence or nonexistence of a charge on each drop (11) as it separates from stream (10) can be controlled by the flow cytometer. This control results in positively charged, negatively charged, and uncharged drops based upon the encapsulated sperm cell. As shown in Figure 3, certain drops are shown as deflected drops (18). These deflected drops (18) are those containing sperm cells (2) differentiated by bearing an X-chromosome or a Y-chromosome. Separated sperm are then deposited in the appropriate collector (17) for later use. See also, International Patent Application PCT/US98/27909, hereby incorporated by reference.

While the above description focuses on the separation of spermatozoa with flow cytometry, separation of X-chromosome bearing spermatozoa and Y-chromosome

bearing spermatozoa based upon the difference in measurable fluorescent emission may also include numerous other technologies such as liquid chromatography, gel electrophoresis, and other technologies that similarly excite the amount of bound fluorochrome to differentiate between X chromosome bearing spermatozoa and the Y chromosome bearing spermatozoa.

Embodiments of the invention can also comprise collecting oocytes from a female mammal. With respect to certain embodiments of the invention, oocytes can be aspirated from the ovaries of the desired female mammal or can be obtained from slaughterhouse ovaries. The oocytes can be matured in TCM199 supplemented with about 10% fetal calf serum plus hormones (15 ng FSH, 1 μ g LH, 1 μ g E_2 /ml) for 22-24 h at 39°C, in about 5% CO₂ in air.

Ten to 15 oocytes can be transferred to a 50 μ l drop of fertilization medium containing non-essential amino acids, such as tyrode albumin lactate pyruvate (TALP) supplemented with non-essential amino acids derived from Eagles Medium, and which can further contain 0.6% bovine serum albumin, 20 μ g heparin/ mL and 5 mM caffeine. Alternately, oocytes can be fertilized in other medium containing non-essential amino acids such as the chemically defined medium described in the journal article entitled "Lowered Oxygen Tension and EDTA Improve Bovine Zygote Development In Chemically Defined Medium", J. Anim. Sci. (1999), or the SOF medium described in the journal article "Successful Culture In-vitro of Sheep and Cattle Ova", J. Reprod. Fertil. 30:493-497 (1972), each journal article hereby incorporated by reference.

After separating or sorting, sperm cells can be washed by centrifugation for about 10 min at 400 g in collection medium (typically Hepes-tyrode albumin lactate pyruvate medium supplemented with 2.0% bovine serum albumin) followed by suspension in the fertilization medium. Thawed, sorted sperm can be prepared by being centrifuged for 20 minutes at 700 g through a Percoll gradient (90%: 45%) for separation of live and dead sperm. The sperm pellet can then be washed with fertilization medium by centrifugation at 400 g for 10 minutes. Sperm can then be added to the fertilization medium to give a concentration of 1-2 million/mL.

Table 1. Cleavage Stage of Oocytes Inseminated with Separated Sperm in Four Different Fertilization Media.

Media	No. oocytes	% cleavage	% 2-cell at 24 h	%8-cell at 72 h
Fert-TALP	168	76	6 ^a	66
Fert-TALP + neaa	176	71	26 ^b	67
CDM	167	89	75 ^c	70
SOF	145	86	49 ^d	69

^{a,b,c,d} Means with different superscripts differ (P<0.05).

Now referring primarily to Table 1, as can be understood, oocytes inseminated with separated spermatozoa in fertilization medium containing non-essential amino acids according to the invention exhibit an increased rate of early development through at least the two cell stage.

Table 2. Embryonic Development and Blastocyst Quality Resulting From Fertilization in Four Different Fertilization Media (averaged over two culture media)

Media	No. oocytes	% blastocysts/oocyte		% Grade 1 blastocysts/ total blastocysts
		Total	D7	
Fert-TALP	326	20	17	52 ^{a,c}
Fert-TALP -aa	221	20	17	68 ^b
CDM	332	22	18	61 ^{b,c}
SOF	321	21	17	64 ^{b,c}

^{a,b,c} Percentages without common superscripts differ (P<.05)

^d Grade 1 indicates blastocysts with a distinct inner cell mass suitable for embryo transfer.

Now referring primarily to Table 2, some embodiments of the invention in which oocytes are fertilized with sorted spermatozoa in fertilization medium containing supplemented non-essential amino acids can exhibit an enhanced quality of embryos. In embodiments of the invention in which oocytes were fertilized in tyrode albumin lactate pyruvate (TALP) supplemented with non-essential amino acids derived from Eagles Medium, and further containing 0.6% bovine serum albumin, 20 µg heparin/ mL and 5 mM caffeine there was a difference (P<.05) in quality of embryos as compared to TALP without non-essential amino acids.

Presumptive zygotes can be removed from culture and placed in chemically-defined medium (CDM-1) as discussed in the Journal Animal Science, 78, 152-157 (2000), hereby incorporated by reference, for 6-7 hours after insemination and cultured for 65-66 hours. Embryos that cleaved were further cultured 96 hours in CDM-2 (further

containing MEM essential and non-essential amino acids and 2.0 mM fructose) containing 0.12 IU insulin/mL. Blastocysts were morphologically graded according to the size of inner cell mass and stained with Giemsa to determine cell numbers on day 7 after insemination.

Now referring primarily to Figure 4, the invention further involves a portable incubation system. Certain embodiments of the invention can comprise a straw (19) having an interior volume between about 0.1 mL and about 0.5 mL into which fertilization medium, and oocytes collected from a female mammal, can be transferred. While the straw (19) could be made of any material compatible with the fertilization medium and the collected oocytes, specific embodiments of the straw (19) can be made of plastic, such as or similar to an artificial insemination straw. The ends of plastic straws can be heat sealed after the fertilization medium and the oocytes are transferred inside.

The invention can further comprise an incubation element (20) configured to encapsulate the straw (19) or a plurality of straws inserted within. In some embodiments of the invention the incubation element (20) can be a glass tube having a single sealable aperture element. The aperture element (21) can be sealed with a cap (22), and in some embodiments the cap (22) and the tube can have spiral threads (23) that can be rotationally mated to close the incubation element (20).

After transfer of a straw (19) or a plurality of straws to the interior volume of the incubation element (20), incubation conditions can be established within. Typical incubation conditions within the interior volume of the incubation element can comprise an atmosphere of five percent carbon dioxide in air and a temperature of about 39°C (37 °C to 41 °C). Once incubation conditions are established within the incubation element, the incubation element (20) can be sealed and the oocytes can then be transported within the incubation element (20).

In some embodiments of the invention, oocytes can be transported to a sperm cell separation facility where the incubation element (20) is unsealed, the straw (19) is unsealed and a plurality of sperm cells (15) from a population separated on the basis of

bearing an X-chromosome or bearing a Y-chromosome can be transferred into the straw (19) containing the oocytes. With respect to some embodiments of the invention a concentration of separated sperm cells (15) can be established of between about 1 million to about 2 million/ mL of the fertilization medium. The straw (19) containing the oocytes and spermatozoa in fertilization medium can then be resealed and transferred back into the incubation element (20). The incubation conditions can be re-established and the incubation element sealed. The incubation element (20) containing a straw or plurality of straws (19) can then be transported. During transport the oocytes can become fertilized. Upon arrival zygotes can be transferred from the straw for further culture.

With respect to certain embodiments of the invention, oocytes can first be inseminated with separated or unseparated spermatozoa in conventional 50 μ l drops and loaded into a 0.25 mL straw or straws (19) within two hours after insemination. Straws (19) can be heat sealed and put into the incubation element (20). The open incubation element containing straws with inseminated oocytes can be equilibrated with 5% carbon dioxide in air at about 39°C for at least one hour and then tightly capped and cultured under the same conditions for between about 18-20 hours.

Again referring primarily to Table 2, fewer oocytes ($P<0.05$) fertilized in Fert-TALP developed to the 2-cell stage by 24 hours than in any other media. Notably, the vast majority of oocytes (75%) fertilized in CDM medium cleaved to 2-cell stage by this time. By 72 hours post-insemination, there was no difference between any of the media, possibly due to the long 8-cell stage cell cycle.

There was no difference between any of the media on rate of development to blastocysts. However, there was a significant difference in quality of embryos between Fert-TALP and Fert-TALP + non-essential amino acids.

Progression of early bovine embryonic development using separated sperm are similar to studies with in-vivo or in-vitro cleavage of oocytes fertilized with unseparated spermatozoa. In the cow the first in-vivo cleavage occurs at 24-28 hours following ovulation, and the first in-vitro cleavage takes place at 24-48 hours after insemination.

Earlier cleavage occurred with oocytes fertilized in CDM, SOF, and Fert-TALP + aa medium than in conventional Fert-TALP medium. This can be because CDM, SOF, and Fert-TALP + non-essential amino acids, all contain non-essential amino acids, which may play a role in how quickly spermatozoa penetrate oocytes, or in the length of the first cell cycle.

As can be easily understood from the foregoing, the basic concepts of the present invention may be embodied in a variety of ways. It involves the staining of spermatozoa, whether fresh spermatozoa or frozen-thawed spermatozoa, separation and isolation techniques which may be used with such stained spermatozoa, as well as devices to accomplish the staining, separation, isolation of such stained spermatozoa into X-chromosome bearing and Y-chromosome bearing populations, and the transportation of maturing oocytes and fertilized oocytes. In this patent application, the staining and separating techniques used with spermatozoa are disclosed as part of the results shown to be achieved by the various devices described and as steps which are inherent to utilization. They are simply the natural result of utilizing the devices as intended and described. In addition, while some devices are disclosed, it should be understood that these not only accomplish certain methods but also can be varied in a number of ways. Importantly, as to all of the foregoing, all of these facets should be understood to be encompassed by this disclosure.

The discussion included in this international Patent Cooperation Treaty patent application is intended to serve as a basic description. The reader should be aware that the specific discussion may not explicitly describe all embodiments possible; many alternatives are implicit. It also may not fully explain the generic nature of the invention and may not explicitly show how each feature or element can actually be representative of a broader function or of a great variety of alternative or equivalent elements. Again, these are implicitly included in this disclosure. Where the invention is described in functionally-oriented terminology, each aspect of the function is accomplished by a device, subroutine, or program. Apparatus claims may not only be included for the devices described, but also method or process claims may be included to address the

functions the invention and each element performs. Neither the description nor the terminology is intended to limit the scope of the claims which now be included.

Further, each of the various elements of the invention and claims may also be achieved in a variety of manners. This disclosure should be understood to encompass each such variation, be it a variation of an embodiment of any apparatus embodiment, a method or process embodiment, or even merely a variation of any element of these. Particularly, it should be understood that as the disclosure relates to elements of the invention, the words for each element may be expressed by equivalent apparatus terms or method terms — even if only the function or result is the same. Such equivalent, broader, or even more generic terms should be considered to be encompassed in the description of each element or action. Such terms can be substituted where desired to make explicit the implicitly broad coverage to which this invention is entitled. As but one example, it should be understood that all actions may be expressed as a means for taking that action or as an element which causes that action. Similarly, each physical element disclosed should be understood to encompass a disclosure of the action which that physical element facilitates. Regarding this last aspect, as but one example, the disclosure of a "sorter" should be understood to encompass disclosure of the act of "sorting" — whether explicitly discussed or not — and, conversely, were there only disclosure of the act of "sorting", such a disclosure should be understood to encompass disclosure of a "sorter" and even a "means for sorting". Such changes and alternative terms are to be understood to be explicitly included in the description.

Additionally, the various combinations and permutations of all elements or applications can be created and presented. All can be done to optimize the design or performance in a specific application.

Any acts of law, statutes, regulations, or rules mentioned in this application for patent: or patents, publications, or other references mentioned in this application for patent are hereby incorporated by reference. Specifically, United States Provisional Patent Application No. 60/253,787, filed November 29, 2000 and United States Provisional Patent Application No. 60/253,785, filed November 29, 2000, are hereby

incorporated by reference including any figures or attachments, and each of references in the following table of references are hereby incorporated by reference.

US Patent Documents

DOCUMENT NO.	DATE	NAME	CLASS	SUBCLASS	FILING DATE
32,350	02/10/87	Bhattacharya			11/22/74
3,687,806	08/29/72	Van den Bovenkamp	195	1.3	11/04/69
3,839,216	08/13/74	Peridisky	356	36	10/03/72
3,894,529	07/15/75	Shrimpton	128	1 R	04/10/69
4,009,260	02/22/77	Ericson	424	105	12/11/74
4,067,965	01/10/78	Bhattacharya	424	105	12/17/75
4,083,957	04/11/78	Lang	424	78	02/04/76
4,085,205	04/18/78	Hancock	424	105	01/24/77
4,092,229	05/30/78	Bhattacharya	204	180 R	10/20/76
4,155,831	05/23/79	Bhattacharya	207	299 R	02/23/78
4,191,749	03/04/80	Bryant	424	105	10/11/77
4,225,405	09/30/80	Lawson	204	180 R	08/16/78
4,276,139	06/30/81	Lawson	204	180 R	10/09/79
4,339,434	07/13/82	Ericson	424	105	08/17/81
4,362,246	12/07/82	Adair	209	3.3	07/14/80
4,448,767	05/15/84	Bryant	424	85	02/15/80
4,474,875	10/02/84	Shrimpton	435	002	08/18/80
4,501,366	02/26/85	Thompson	209	556	12/14/82
4,511,661	04/16/85	Goldberg	436	503	12/30/83
4,605,538	08/12/86	Shrimpton	424	561	04/20/84
4,660,971	04/28/87	Sage et al.	356	39	03/03/84
4,680,258	07/14/87	Hammerling et al	435	7	08/09/83
4,673,288	06/16/87	Thomas et al.			
4,683,195	07/28/87	Mullis et al			
4,683,202	07/28/87	Mullis			
4,698,142	10/06/87	Muroi et al	204	182.3	07/31/85
4,749,458	06/07/88	Muroi et al	204	182.3	03/02/87
4,790,653	12/13/88	North, Jr.			
4,988,619	01/29/91	Pinkel	435	30	11/30/87
4,999,283	03/12/91	Zavos et al	435	2	08/18/89
5,021,244	06/04/91	Spaulding	424	561	05/12/89
5,055,393	10/08/91	Kwoh et al			
5,135,759	08/04/92	Johnson	424	561	04/26/91
5,346,990	09/13/94	Spaulding	530	350	03/12/91
5,371,585	12/06/94	Morgan et al.	356	246	11/10/92
5,437,987	08/01/95	Ten et al			
5,439,362	08/08/95	Spaulding	424	185.1	07/25/94
5,461,145	10/24/95	Kudo et al			
5,466,572	11/14/95	Sanki et al.	435	2	04/25/94
5,480,774					
5,483,469	01/09/96	Van den Engh et al.	364	555	08/02/93
5,494,795	2/27/96	Guerry et al.	435	6	5/5/93
5,503,994	04/02/96	Shear et al.	436	90	10/08/93
5,578,449	11/26/96	Frach et al.	435	6	4/20/95
5,514,537	05/07/96	Chandler	435	002	11/28/94

5,589,457	12/31/96	Wiltbank	514	12	07-03-95
5,602,039	02/11/97	Van den Engh	436	164	10/14/94
5,602,349	02/11/97	Van den Engh	73	864.85	10/14/94
5,622,820	4/11/97	Rossi	435	5	11/3/94
5,641,457	03/09/99	Tomiyama et al.	250	207	06/16/97
5,643,796	07/01/97	Van den Engh et al.	436	50	10/14/94
5,660,997	08/26/97	Spaulding	435	7.21	06/07/95
5,690,895	11/25/97	Matsumoto et al.	422	73	12/06/96
5,700,692	12/23/97	Sweet	436	50	09/27/94
5,726,364	03/10/98	Van den Engh	73	864.85	02/10/97
5,819,948	10/13/98	Van den Engh	209	158	08/21/97
5,876,942	3/2/99	Cheng et al.	435	6	7/24/97
5,880,457	03/09/99	Tomiyama et al.	250	207	06/16/97
5,985,216	11/16/99	Rena, et al.	422	073	07/24/97
6,071,689	06/06/00	Seidel et al.	435	2	01/29/98

Foreign Documents

DOCUMENT NO	DATE	COUNTRY
WO 96/12171	10/13/95	United States
WO 98/34094	06/08/98	NZ
WO 99/05504	07/24/98	US
WO 99/33956	08/07/99	US
WO 99/38883	05/08/99	US
WO 99/42810	08/26/99	US
WO 00/06193	10/02/00	US

Other Reference Documents

Roser, J.F., Evans, J.W., Kiefer, D.P., Neeley, D.P. and Pacheco, C.A. 1980. Reproductive efficiency in mares with anti-hCG antibodies. Proc 9 th Int. Congr. Artira. Repro. and A.I. 4:627. abstr.
"Applying Semen Sexing Technology to the AI Industry", National Association of Animal Breeders, September 2000, pp. 1-16
"Sexed Semen Offers Faster Genetic Gain", Farming News, Livestock Supplement, February 1997, p. 28.
Akhtar, S., et al., "Prevalence of Five Stereotypes of Bluetongue Virus in a Rambouillet Sheep Flock in Pakistan", Veterinary record 136, 1995, p. 495.
Akhtar, S., et al., "Sex Preselected in Cattle: a Field Trial", Veterinary Record 136, 1995, p. 495-496.
Aldrich, S. L., Berger, L.L., Reiling, B.A., Keyler, D.I., and Nagh, T.G.. 1995. "Parturition and periparturient reproductive and metabolic hormone concentration in prenatally androgenized beefheifers", J. Anim. Sci. 73:3712.
Amann, R. P. "Issues affecting commercialization of sexed sperm". Therio: 52:1441, 1999
Amann, R.P. et al, "Prospects For Sexing Mammalian Sperm," Colorado Associated University Press, Animal Reproduction Laboratory College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, 80523, 1982
American Meat Science Association in cooperation with National Livestock and Meat Board. "Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meat", 1995
Amos, E.A. and Gelaye, S. 1996. Biotechnological advances in goat reproduction. J. Anim. Sci. 75(2):578-585.
Andersen, V.K., Aamdal, J. and Fougner, J.A. 1973. Intrauterine und tiefervikale Insemination mit Gefriersperma beim Schaf. Zuchthygiene. 8:113-118.
Begley, C. P. 1993. Nutritional management of replacement beef heifers -A review. J. Anim. Sci. 71:3155-3163.
Bailey, C. M., Reid, C.R., Ringkob, T.P., Koh, Y.O., and Foote, W.D. "Nulliparous versus primiparous crossbred females for beef." J. Anim. Sci. 69:1403., 1991

- Baker, R.D., Dziuk, P.J. and Norton, H.W. 1968. Effect of volume of semen, number of sperm and drugs on transport of sperm in artificially inseminated gilts. *J. Anim. Sci.* 27:88-93.
- Barnes, F.L. and Eyestone, W.H. "Early Cleavage and the Maternal Zygotic Transition in Bovine Embryos", *Theriogenology*, Vol. 33, No. 1, January 1990, pp. 141-149
- Becker, S.E. and Johnson, A.L. 1992. Effects of gonadotropin releasing hormones infused in a pulsatile or continuous fashion on serum gonadotropin concentrations and ovulation in the mare. *J. Anim. Sci.* 70:1208-1215.
- Bedford, S. J. and Hinrichs, K. 1994. The effect of insemination volume on pregnancy rates of pony mares. *Theriogenology* 42:571-578.
- Bellows, R. A., Short, R.E., Anderson, D.C., Knapp, B.W., and Pabniah, O.F. "Cause and effect relationships associated with calving difficulty and calfbirth weight", *J. Anim. Sci.* 33:407, 1971
- Berardinelli, J. G., R. A. Dailey, R. L. Butcher, and E. K. Inskeep. "Source of progesterone prior to puberty in beef heifers". *J. Anim. Sci.* 49:1276., 1979
- Berger, G.S. 1987. Intratubal insemination. *Fert. Steril.* 48:328-330.
- Bergfeld, E. G., Kojima, F.N., Cupp, A.S., Wehman, M.E., Peters, K.T., Garciawinder, M., and Kinder, J.E. "Ovarian follicular development in prepubertal heifers is influenced by level of dietary energy-intake", *Bio. of Repro.* 51:1051, 1994
- Berry, B. W., Smith, G.C., and Carpenter, J., "Beef carcass maturity indicators and palatability attributes", *J. Anim. Sci.* 38:507, 1974
- Beyhan, Z., et al., "Sexual Dimorphism in IVF Bovine Embryos Produced by Sperm Sorted by High Speed Flow Cytometry", *Theriogenology* 49, 1998, p. 359.
- Blanchard, T. and Dickson, V., "Stallion Management", *The Veterinary Clinics of North America, Equine Practice*, Vol. 8, No. 1, April 1992, pp 207 - 218.
- Bond, J., et al., "Growth and carcass traits of open beef heifers versus beef heifers that have calved", *Nutrition Reports International* 34:621. 1986
- Bouque, C. V., et al., "Beef-production with maiden and once-calved heifers", *Livestock Prod. Sci.* 7:121. 1980
- Bourdon, R. M. and J. S. Brinks. "Simulated efficiency of range beef-production". Culling strategies and nontraditional management-systems. *J. Anim. Sci.* 65:963. 1987
- Bracher, V. and Allen, W.R., "Videoscopic Examination of the Mare's Uterus: Findings in Normal Fertile Mares", *Equine Veterinary Journal*, Vol. 24 (1992), pp. 274-278
- Braserton, W.E. and McShan, W.H. 1970. "Purification and properties of follicle stimulating and luteinizing hormones from horse pituitary glands", *Arch. Biochem. Biophys.* 139:45-48.
- Brethour, J. R., "The single-calfheifer system", *Kans. Agric. Sta. Rep. Prog.* 570. 1989
- Bristol, S.P. 1982. Breeding behavior of a stallion at pasture with 20 mares in synchronized oestrus. *J. Reprod. Fert. Suppl.* 32:71.
- Brookes, A. J. and Obyrne, M., "Use of cow-heifers in beef production", *J. of the Royal Agricultural Society of England* 126:30. 1965
- Buchanan, B.R., et al, "Insemination of Mares with Low Numbers of Either Unsexed or Sexed Spermatozoa", *Theriogenology*, Vol. 53, pp 1333-1344, (2000)
- Burns, P. D. and Spitzer, J.C., "Influence of bio-stimulation on reproduction in postpartum beef-cows", *J. Anim. Sci.* 70:358. 1992
- Burwash, L.D., Pickett, B.W., Voss, J.L. and Back, D.G. 1974. "Relationship of duration of estrus to pregnancy rate in normally cycling, non-lactating mares" *J.A.V.M.A.* 165:714-716.
- Byerley, D. J., et al., "Pregnancy rates of beef heifers bred either on puberal or 3rd estrus". *J. Anim. Sci.* 65:645. 1987
- Caslick, E.A., "The Vulva and the Vulvo-vaginal Orifice and its Relation to Genital Health of the Thoroughbred Mare", *Cornell Veterinarian*, Vol. 27, 1937, pp. 178-187
- Cart, et al., "Assessment of Ram and Boar Spermatozoa During Cell-Sorting by Flow Cytometry", *Reproduction Dom Animal*, Vol. 32, 1997, pp 251-258.
- Cart, S.L., et al., "Birth of a Male Lamb Derived from an In Vitro Matured Oocyte Fertilized by Intracytoplasmic Injection of a Single Presumptive Male Sperm", *Veterinary Record* 139, 1996, pp. 494-495.
- Chin, W.W. and Boime, I. 1990. In: *Glycoprotein Hormones. Serona Symp.* Norwell, MA. pp. 19-20
- Chung, Y.G., Schenk, J.L., Herickhoff, L.A. and Seidel, G.E. Jr. 1998. Artificial insemination of superovulated heifers with 600,000 sexed sperm. *J. Anim. Sci. Suppl.* 1. 836:215. abstr.
- Clement, F., Vincent, P., Mahla, R., Meriaux, J.C. and Palmer, E. 1998. Which insemination fertilizes when

several successive inseminations are performed before ovulation. 7th Int. Symp. Eq. Repr. 151. abstr.

Coleou, J., et al., "Essai de velage tres precoc de genisses en vue de la production de viande." Essai Vauz/ Auro no.50, programme USFGC-INAPG-ITFC. 1974

Cran, D.G., et al., "Production of Bovine Calves Following Separation of X- and Y- Chromosome Bearing Sperm and In Vitro Fertilisation", *Veterinary Record* 132, 1993, pp. 40-41.

Cran, D.G., et al., "Production of Lambs by Low Dose Intrauterine Insemination with Flow Cytometrically Sorted and Unsorted Semen", *Theriogenology* 47, 1997, p. 267.

Crowley, J. P. The facts of once-bred heifer production. (Ed) J.B. Owens. The maiden female-a means of increasing meat production. School of Agric., Univ. of Aberdeen, Scotland. 1973

Curran, S. 1998. In: *Equine Diagnostic Ultrasonography. Fetal gender determination*. Rantanen & McKinnon. 1st Ed. Williams and Wilkins. pp. 165-169.

Day, B.N., Abeydeera, L.R., Johnson, L.A., Welch, G.R., Wang, W.H., Cantley, T.C. and Rieks, A. 1998. Birth of piglets preselected for gender following in vitro fertilization of in vitro matured pig oocytes by X and Y bearing spermatozoa sorted by high speed flow cytometry. *Theriogenology*. 49(1):360. abstr.

Dean, P.N., Pinkel, D. and Mendelsohn, M.L. 1978. Hydrodynamic orientation of spermatozoa heads for flow cytometry. *Biophys. J.* 23:7-13.

Demick, D.S., Voss, J.L. and Pickert, B.W. 1976. Effect of cooling, storage, glycerization and spermatozoal numbers on equine fertility. *J. Anim. Sci.* 43:633-637.

DenDaas, J.H.G., De Jong, G., Lansbergen, L.M.T.B. and Van Wageningen-De Leeuw, A.M. 1998. The relationship between the number of spermatozoa inseminated and the reproductive efficiency of dairy bulls. *J. Dairy Sci.* 81: 1714-1723.

Denham, A. "In-vitro studies on sandhill range forage as related to cattle preference", M.S. Thesis. 1965. Colorado State University.

Deutcher, G. H. "Extending interval from seventeen to nineteen days in the megestrol acetate-prostaglandin estrous synchronization program for heifers". *The Professional Animal Scientist* 16:164. 2000

"Diagnostic Products Corporation. Coat-A-Count", *Progesterone.com*. 1998.

Dikeman, M. B. "Cattle production systems to meet future consumer demands. *J. Anim. Sci.* 59:1631, 1984

Dinnyes, A., et al., "Timing of the First Cleavage Post-insemination Affects Cryosurvival of In Vitro-produced Bovine Blastocysts", *Molec Reprod Develop* 53, 1999, pp 318-324.

Donaldson, L. R., "Effect of Insemination Regimen on Embryo Production in Superovulated Cows", *The Veterinary Record*, July 13, 1985, pp. 35-37

Donoghue, A.M., Byers, A.P., Johnston, L.A., Armstrong, D.L. and Wildt, D.E. 1996. Timing of ovulation after gonadotropin induction and its importance to successful intrauterine insemination in the tiger (*Panthera tigris*). *J. Reprod. Fert.* 107:53-58.

Douglas, R.H. 1979. Review of superovulation and embryo transfer in the equine. *Theriogenology*. 11:33-46.

Douglas, R.H., Nutt, L. and Ginther, O.J. 1974. Induction of ovulation and multiple ovulation on seasonally-anovulatory mares with equine pituitary fractions. *Theriogenology*. 2(6): 133-142.

Doyle, S. P., et al. "Artificial insemination of lactating Angus cows with sexed semen". *Proc. Western Sect. Am.Soc.Anim. Sci.* 50:203. 1999

Duchamp, G., Bour, B., Combarrous, Y. and Palmer, E. 1987. Alternative solutions to hCG induction of ovulation in the mare. *J. Reprod. Fert. Suppl.* 35:221-228.

Evans, M.J. and Irvine, C.H.G. 1977. Induction of follicular development, maturation and ovulation by gonadotropin releasing hormone administration to acyclic mares. *Bio. Reprod.* 16:452-462.

Ferrell, C. L. and T. G. Jenkins. "Energy-Utilization by Mature, nonpregnant, nonlactating cows of different types" *J. Anim. Sci.* 58:234. 1984

Ferrell, C. L. "Effects of post-weaning rate of gain on onset of puberty and productive performance of heifers of different breeds. *J. Anim. Sci.* 55:1272. 1982

Field, R. A., et al., "Bone-ossification and carcass characteristics of wethers given silastic implants containing estradiol". *J. Anim. Sci.* 68:3663-3668. 1990

Field, R., R. et al., "Growth, carcass, and tenderness characteristics of virgin, spayed, and single-calf heifers.", *J. Anim. Sci.* 74:2178. 1996

Fitzgerald, B.P., Peterson, K.D. and Silvia, P.J. 1993. Effect of constant administration of a gonadotropin-releasing hormone agonist on reproductive activity in mares: Preliminary evidence on suppression of ovulation during the breeding season. *Am. J. Vet. Res.* 54:1746-1751.

Fluharty, F. L., et al., "Effect of weaning and diet on growth of calves." Research and Reviews. The Ohio State University Department of Animal Sciences. 1996
Fluharty, F.L., et al., "Effects of Age at Weaning and Diet on Growth of Calves", Ohio Agri. Res. and Dev. Circular, 1996. 156: 29.
Foulkes, J.A., Stewart, D.L. and Herbert, C.N. 1977. Artificial insemination of cattle using varying numbers of spermatozoa. Vet. Rec. 101:205.
Fugger, E.F., "Clinical Experience with Flow Cytometric Separation of Human X- and Y- Chromosome Bearing Sperm", Theriogenology, Vol. 52, pp. 1435-1440 (1999)
Fulwyler, M.J. 1965. Electronic separation of biological cells by volume. Science. 150:910.
Fulwyler, M.J. 1977. Hydrodynamic orientation of cells. J Histochem. Cytochem. 25:781-783.
Seidel, G.E. Jr., "Artificial Insemination With X-and Y-Bearing Bovine Sperm", Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO; Germplasm and Gamete Physiology Lab, ARS, USDA, Beltsville, MD; Atlantic Breeders Coop, Lancaster, PA; DUO Dairy, Loveland, CO, USA January 1996.
Gamer, D.L., Gledhill, B.L., Pinkel, D., Lake, S., Stephenson, D., Van Dilla, M.A. and Johnson, L.A. 1983. "Quantification of the X and Y chromosome-bearing spermatozoa of domestic animals by flow cytometry". Biol. Reprod. 28:312-321.
Ginther, O.J. 1983. Sexual behavior following introduction of a stallion into a group of mares. Theriogenology. 19:877.
Ginther, O.J. 1992. In: Reproductive Biology of the Mare. (2 nd Ed.) Equiservices, Cross Plains, WI.
Gledhill, B.L. 1988. Gender preselection: historical, technical and ethical perspective. Semin Reprod. Endocrinol. 6:385-395.
Gombe, S. and Hansel, W. "Plasma luteinizing-hormone (LH) and progesterone levels in heifers on restricted energy intakes." J. Anim. Sci. 37:728. 1973
Gourley, D.D. and Riess, R.L. 1990. Laparoscopic artificial insemination in sheep. Vet. Clin. N. Amer: Food Anim. Prac. 6(3):615-633.
Gravert, H. O., "Genetic Aspects of Early Calving." In: J.C. Taylor (Ed.) The early calving of heifers and it's impact on beef production. 59. 1975
Gregory, K. E., et al., "Characterization of biological types of cattle III. 2." Growth-rate and puberty in females. J. Anim. Sci. 49:461. 1979
Grimes, L. F. and T. B. Turner. "Early weaning of fall born calves II." Post weaning performance of early and normal-weaned calves. I. Prod. Agric. 4:168. 1991
Grondehl, C., et al, "In Vitro Production of Equine Embryos", Biology of Reproduction, Monograph Series I, pp. 299-307 (1995)
Guilou, F. and Combarnous, Y. 1983. Purification of equine gonadotropins and comparative study of their acid-dissociation and receptor-binding specificity. Biochem. Biophys. Acta. 755:229-236.
Gurnsey, M.P., and Johnson, L.A., "Recent improvements in efficiency of flow cytometric sorting of X and Y-chromosome bearing sperm of domestic animals: a review", 1998, New Zealand Society of Animal Protection, three pages.
Hall, J. B., et al., "Effect of age and pattern of gain on induction of puberty with a progestin in beef heifers." J. Anim. Sci. 75:1606. 1997
Hamano, K., et al., "Gender Preselection in Cattle with Intracytoplasmically Injected, Flow Cytometrically Sorted Sperm Heads", biology of Reproduction 60, 1999, pp. 1194-1197.
Harrison, L.A., Squires, E.L. and McKinnon, A.O. 1991. Comparison of hCG, busarelin and luproliol for induction of ovulation in cycling mares. Eq. Vet. Sci. 3:163-166.
Hart, F. J. "System of production of bee from once calved heifers." In: J.C. Taylor (Ed.) The early calving of heifers and it's impact on beef production. 123. 1975
Hawk, H.W., et al., "Fertilization Rates in Superovulating Cows After Deposition of Semen on the Infundibulum Near the Uterotubal Junction or After Insemination with High Numbers of Sperm", XP-002103478, Theriogenology, May 1988, Vol. 29, No. 5, pp 1131-1142.
Hemlesmeyer, G. N., et al. "Effects of lactation and prenatal androgenization on the performance, carcass composition and longissimus muscle sensory characteristics of heifers in the single-calf-heifer system." The Professional Animal Scientist 15:14. 1999
Hennegmeyer, G. N., et al. "Effects of prenatal androgenization and implantation on the performance and carcass

composition of lactating heifers in the single-calfheifer system." <i>The Professional Animal Scientist</i> 15:173. 1999
Hilton, G. G., et al., "An evaluation of current and alternative systems for quality grading carcasses of mature slaughter cows." <i>J. Anim. Sci.</i> 76:2094. 1998
Ho, L., et al., "Influence of gender, breed and age on maturity characteristics of sheep." <i>J. Anim. Sci.</i> 67:2460-2470. 1989
Hofferer, S., Lecompte, F., Magallon, T., Palmer, E. and Combarnous, Y. 1993. Induction of ovulation and superovulation in mares using equine LH and FSH separated by hydrophobic interaction chromatography. <i>J. Reprod. Fert.</i> 98:597-602.
Hohenboken, W. D. "Applications of sexed semen in cattle production." <i>Therio</i> 52:1421. 1999
Holtan, D.W., Douglas, R.H. and Givther, O.J. 1977. Estrus, ovulation and conception following synchronization with progesterone, prostaglandin F2 α and human chorionic gonadotropin in pony mares. <i>J. Anim. Sci.</i> 44:431-437.
Householder, D.D., Pickett, B.W., Voss, J.L. and Olar, T.T. 1981. Effect of extender, number of spermatozoa and hCG on equine fertility. <i>J. Equine Vet. Sci.</i> 1:9-13.
Howard, J.G., Bush, M., Morton, C., Morton, F., Wentzel, K. and Wildt, D.E. 1991. Comparative semen cryopreservation in ferrets (<i>Mustela putorius furo</i>) and pregnancies after laparoscopic intrauterine insemination with frozen-thawed spermatozoa. <i>J. Reprod. Fert.</i> 92:109-118.
Howard, J.G., Roth, T.L., Byers, A.P., Swanson, W.F. and Wildt, D.E. 1997. Sensivity to exogenous gonadotropins for ovulation and laparoscopic artificial insemination in the cheetah and clouded leopard. <i>Biol. Reprod.</i> 56:1059-1068.
Hunter, R.H.F. 1980. Transport and storage of spermatozoa in the female reproductive tract. <i>Proc 4th Int. Congr. Artira. Repro. and A.I.</i> 9:227-233.
Hyland, J.H., Ainsworth, C.G.V. and Langford, D.A. 1988. Gonadotropin-releasing hormone (GnRH) delivered by continuous infusion induces fertile estrus in mares during seasonal anovulation. <i>Proc. Amer. Assoc. Eq. Prac.</i> 181-190.
Irvine, C.H.G. and Alexander, S.L. 1993. <i>In: Equine Reproduction</i> . Edited by McKinnon and Voss. Lea and Febiger. Philadelphia, London. pp. 37.
Jafar, et al., "Sex Selection in Mammals: A Review", <i>Theriogenology</i> , vol. 46, 1996, pp 191-200.
Jarrige, R. "Age of cows at first calving in France." J.C. Taylor (Ed.) <i>The early calving of heifers and its impact on beef production</i> . 10. 1975
Jasko, D.J., Martin, J.M. and Squires, E.L. 1992. Effect of volume and concentration of spermatozoa on embryo recovery in mares. <i>Theriogenology</i> . 37:1233-1239
Johnson L.A., et al., 1987. Flow cytometry of X- and Y- chromosome bearing sperm for DNA using an improved preparation method and staining with Hoechst 333-42. <i>Garnets Research</i> 17: 203-212
Johnson, "Gender preselection in Mammals: An overview", <i>Dtsch. Tierarztl. Wschr.</i> , Vol. 103, Aug./Sep. 1996, pp 288-291.
Johnson, A.L. 1986. Pulsatile release of gonadotropin releasing hormone advances ovulation in cycling mares. <i>Biol. Reprod.</i> 35:1123E 1130.
Johnson, A.L. and Becker, S.E. 1988. Use of gonadotropin-releasing hormone (GnRH) treatment to induce multiple ovulations in the anestrous mare. <i>Eq. Vet. Sci.</i> 8:130-134.
Johnson, L., "Sex Preselection by Flow Cytometric Separation of X and Y Chromosome-Bearing Sperm Based on DNA Difference: a Review", <i>Reproduction and Fertilization Development</i> 7, 1995, pp. 893-903.
Johnson, L., "Successful Gender Preselection in Farm Animals", <i>Agricultural Biotechnology</i> , 1998, pp. 439-452.
Johnson, L.A. 1988. Flow cytometric determination of spermatozoa sex ratio in semen purportedly enriched for X or Y bearing spermatozoa. <i>Theriogenology</i> . 29:265. abstr.
Johnson, L.A. 1992. Gender preselection in domestic animals using flow cytometrically sorted sperm. <i>J Anim. Sci. Suppl</i> 1.70:8-18.
Johnson, L.A. 1994. Isolation of X- and Y-bearing spermatozoa for sex preselection. <i>In: Oxford Reviews of Reproductive Biology</i> . Ed. HH Charlton. Oxford University Press. 303-326.
Johnson, L.A. 1995. Sex preselection by flow cytometric separation of X and Y chromosome bearing spermatozoa based on DNA difference: a review. <i>Reprod. Fert. Dev.</i> 7:893-903.
Johnson, L.A. and Schulman, J.D. 1994. The safety of sperm selection by flow cytometry. <i>Hum. Reprod.</i> 9(5):758.
Johnson, L.A., "Sex preselection in swine: altered sex ratios in offspring following surgical insemination of flow-

sorted X- and Y-bearing sperm", <i>Reprod. Domest. Anim.</i> 26:309-314, 1991
Johnson, L.A., and Pinkel, D., "Modification of a Laser-Based flow Cytometer for High-Resolution DNA Analysis of Mammalian Spermatozoa", <i>Cytometry</i> 7, 1986, pp 268 - 273.
Johnson, L.A., et al., "Sex Preselection in Rabbit Live Births from X and Y Sperm Separated by DNA and Cell Sorting", <i>Exceptional Paper-Rapid Publication</i> , XP-002103476, <i>Biology of Reproduction</i> 41, 199-203, 1989, pp 199-203.
Johnson, L.A., et al., 1994. Improved flow sorting resolution of X- and Y- chromosome bearing viable sperm separation using dual staining and dead cell gating. <i>Cytometry</i> 17 (suppl 7):83.
Johnson, L.A., Flook, J.P., Look, M.V. and Pinkel, D. 1987b. Flow sorting of X and Y chromosome bearing spermatozoa into two populations. <i>Gam. Res.</i> 16:203-212.
Johnson, L.A., Welch, G.R., Rena, W. and Dobrinsky, J.R. 1998. Enhanced flow cytometric sorting of transnational X and Y sperm: high speed sorting and orienting no77.1e for artificial insemination. <i>Theriogenology</i> . 49(1):361. abstr.
Joseph, R. L. "Carcass composition and meat quality in once calved heifers." In: J.C. Taylor (Ed.) <i>The early calving offheifers and its impact on beef production</i> . 143. 1975
Joseph, R. L. and J. P. Crowley. "Meat quality of once-calved heifers." <i>Irish J. of Agric. Research</i> 10:281. 1971
Kachel, V., et al., A Uniform Lateral Orientation, Caused by Flow Forces, of Flat Particles in Flow-Through Systems@, <i>The Journal of Histochemistry and Cytochemistry</i> , 1997, Vol. 25, No. 7, pp 774 -780.
Kanayama, K., Sankai, T., Nariaik, K., Endo, T. and Sakuma, Y. 1992b. Pregnancy by means of tubal insemination and subsequent spontaneous pregnancy in rabbits. <i>J. Int. Med. Res.</i> 20:401-405.
Karabinus, et al., "Effects of Egg Yolk-Citrate and Milk Extenders on Chromatin Structured Viability of Cryopreserved Bull Sperm", <i>Journal of Dairy Science</i> , Vol. 74, No. 11, 1999, pp 3836-3848.
Keeling, P. C. B. M. S. T. G. D. I. a. P. W. J., "A modeling study of once-bred heifer beef production." <i>Proceedings of the New Zealand Society of Animal Production</i> . 51. 1991
Kilicarslan, M.R., Horuz, H., Senunver, S.C., Konuk, S.C., Tek, C. and Carioglu, B. 1996. Effect of GnRH and hCG on ovulation and pregnancy in mares. <i>Vet. Rec.</i> 139:119-120.
Kinder, J. E., et al. "Endocrine basis for puberty in heifers and ewes." <i>J. Repro. and Fertility</i> 393. 1995
Klindt, J. and J. D. Crousa. "Effect of ovariectomy and ovariectomy with ovarian auto transplantation on feedlot performance and carcass characteristics offheifers." <i>J. Anim. Sci.</i> 68:3481. 1990
Klosterman, E. W. and C. F. Parker. "Effect of size, breed and sex upon feed efficiency in beef cattle." <i>North Central Regional Research Publication</i> 235, <i>Ohio Agric. Research and Development Center</i> 1090:3. 1976
Kniffen, D. M., Wagner, W.R., and Lewis, P.E. "Effects of long-term estrogen implants in beef heifers." <i>J. Anim. Sci.</i> 77:2886. 1999
Koch, R. M., et al., "Characterization of biological types of cattle -Cycle-II 3." <i>Carcass composition, quality and palatability</i> . <i>J. Anim. Sci.</i> 49:448. 1919
Lapin, D.R. and Ginther, O.J. 1977. Induction of ovulation and multiple ovulations in seasonally anovulatory and ovulatory mares with an equine pituitary extract. <i>J. Anim. Sci.</i> 44:834-842.
Laster, D. B., "Factors affecting dystocia and effects of dystocia on subsequent reproduction in beef-cattle." <i>J. Anim. Sci.</i> 36:695. 1973
Lawrenz, R. 1985. Preliminary results of non-surgical intrauterine insemination of sheep with thawed frozen semen. <i>J S Afr. Vet. Assoc.</i> 56(2):61-63.
Levinson, G., et al, 1995. DNA-based X-enriched sperm separation as an adjunct to preimplantation genetic testing for the preparation of X-linked disease. <i>Mol. Human Reprod.</i> 10:979-982.
Lindsey, A., et al., A Hysteroscopic Insemination of Mares with Nonfrozen Low-dose Unsexed or Sex-sorted Spermatozoa@, currently unpublished, pp. 1-15.
Linge, F. 1972. Faltföroök med djupfrost sperma (field trials with frozen sperm). <i>Farkotsel.</i> 52:12-13.
Loneragan, P., et al., "Effect of Time Interval from Insemination to First Cleavage on the Development of Bovine Embryos In Vitro and In Vivo", <i>Theriogenology</i> , 1999, p. 326
Long, C.R., Rath, D., Welch, G.R., Schreier, L.L., Dobrinsky, J.R. and Johnson, L.A. 1998. AIn vitro production of porcine embryos from semen sorted for sex with a high speed cell sorter: comparison of two fertilization media@. <i>Theriogenology</i> . 49(1):363. abstr.
Loy, R.G. and Hughes, J.P. 1965. The effects of human chorionic gonadotropin on ovulation, length of estrus, and fertility in the mare. <i>Cornell Vet.</i> 56:41-50.
Lu, K.H., et al., "In Vitro Fertilization with Flow-Cytometrically-Sorted Bovine Sperm", <i>Theriogenology</i> 52.

1999, pp. 1393-1405.

Lynch, I. M., et al., "Influence of timing of gain on growth and reproductive performances of beef replacement heifers." *J. Anim. Sci.* 75:1715. 1997

Macmillan, K.L. and A.M. Day, "Prostaglandin F_{2a} - A Fertility Drug in Dairy Cattle?", *Ruakura Animal Research Station, Private Bag, Hamilton, New Zealand, Theriogenology*, September 1982, Vol. 18 No. 3, pages 245-253

Martin, A. H., et al., "Characteristics of youthful beef carcasses in relation to weight, age and sex 3. meat quality attributes." *Canadian J. Anim. Sci.* 51:305. 1971

Martin, L. C., J. S. Brinks, R. M. Bourdon, and L. V. Cundiff. "Genetic-effects on beef heifer puberty and subsequent reproduction." *J. Anim. Sci.* 70:4006. 1992

Matuda, Y. and Toberi, L. 1988. Chromosomal analysis in mouse eggs fertilized *in vitro* with sperm exposed to ultraviolet light (UV) and methyl and ethyl methanesulfonate (MMS and EMS). *Mutat. Res.* 198:131-144.

Matulis, R. J., F. K. McKeith, D. B. Faulkner, L. L. Berger, and P. George. "Growth and carcass characteristics of cull cows after different times-on-feed." *J. Anim. Sci.* 65:669. 1987

Mauleon, P. "Recent research related to the physiology of puberty." Commission of the European Communities. *The early calving of heifers and its impact on beef production.* 1975

Maxwell, W. and Johnson, L., "Chlortetracycline Analysis of Boar Spermatozoa after Incubation, Flow Cytometric Sorting, Cooling, or Cryopreservation", *Molecular Reproduction and Development* 46, 1997, pp. 408-418.

Maxwell, W.M.C., Evans, G., Rhodes, S.L., Hillard, M.A. and Bindon, B.M. 1993. Fertility of Superovulated Ewes after Intrauterine or Oviductal Insemination with Low Numbers of Fresh or Frozen-Thawed Spermatozoa. *Reprod. Fertil. Dev.* 5:57-63.

Mcconnick, R. J. "The flexibility of the collagen compartment of muscle." *Meat Sci.* 36:79. 1994

McCua, P.M. 1996. Superovulation. *Vet. Clin. N. Amer. Eq. Prac.* 12:1-11.

McCua, P.M., Fleury, J.J., Denniston, D.J., Graham, J.K. and Squires, E.L. 1997. Oviductal insemination in the mare. 7th Int Symp. Eq. Reprod. 133. abstr.

McDonald, L.E. 1988. Hormones of the pituitary gland. In: *Veterinary Pharmacology and Therapeutics*. 6th ed. Edited by N.H. Booth and L.E. McDonald. Ames, Iowa State Univ. Press. pp. 590.

McKenna, T., Lenz, R.W., Fenton, S.E. and Ax, R.L. 1990. Nonreturn rates of dairy cattle following uterine body or cervical insemination. *J. Dairy Sci.* 73:1779-1783.

McKinnin, A. and Voss, J., "Equine Reproduction", Lea & Febiger, Philadelphia, 1993, pp 291, 299 - 302, 345 - 348, 739 - 797.

McKinnon, A. et al, 1993. Predictable ovulation in mares treated with an implant of the GnRH analogue deslorelin. *Eq. Vet. J.* 25:321-323.

McKinnon, A.O. et al, 1996. Repeated use of a GnRH analogue deslorelin (Ovuplant) for hastening ovulation in the transitional mare. *Eq. Vet. J.* 29:153-155.

McNurt, et al., "Flow Cytometric Sorting of Sperm: Influence on Fertilization and Embryo/Fetal Development in the Rabbit", *Molecular Reproduction and Development*, Vol. 43, 1996, pp 261-267.

Meilgaard, M., G. V. Civile, and B. T. Carr. "Sensor Evaluation Techniques." CRC Press Inc., Boca Raton, FL. 1991

Meinert, C., et al., "Advancing the time of ovulation in the mare with a short-term implant releasing the GnRH analogue deslorelin", *Equine Veterinary Journal*, 25, 1993, pp 65 - 68.

Merton, J., et al., "Effect of Flow Cytometrically Sorted Frozen/Thawed Semen on Success Rate of In Vitro Bovine Embryo Production", *Theriogenology* 47, 1997, pp. 295.

Meyers, P.J., Bowman, T., Blodgett, G., Conboy, H.S., Gimenez, T., Reid, M.P., Taylor, B.C., Thayer, J., Joehle, W. and Trigg, T.E. 1997. Use of the GnRH analogue, deslorelin acetate, in a slow release implant to accelerate ovulation in oestrous mares. *Vet. Rec.* 140:249-252.

Michalek, Charles, "Beef A.I. Facilities that work", *Proc. Fifth N.A.A.B Tech. Conf. A.I. Reprod.* Columbia, MO. pp. 20-22.

Michel, T.H., Rosedale, P.D. and Cash, R.S.G. 1986. Efficacy of human chorionic gonadotrophin and gonadotrophin releasing hormone for hastening ovulation in Thoroughbred mares. *Eq. Vet. J.* 6:438-442.

Miller, S.J. 1986. *Artificial Breeding Techniques in Sheep*. In Morrow, D.A. (ed): *Current Therapy in Theriogenology* 2. Philadelphia, WB Saunders.

Mirskaja, L.M. and Petrapavlovskii, V.V. 1937. The reproduction of normal duration of heat in the mare by the

administration of Proln. Probl. Zivotn. Anim. Breed. Abstr. 5:387.
Moe, P. W., H. F. Tyrrell, and W. P. Flatt. "Energetics of body tissue mobilization." <i>J. of Dairy Sci.</i> 34:548.
Molinia, F.C., Gibson, R.J., Brown, A.M., Glazier, A.M. and Rodger, J.C. 1998. Successful fertilization after superovulation and laparoscopic intrauterine insemination of the brushtail possum, <i>Trichosurus vulpecula</i> , and tamar wallaby, <i>Macropus eugenii</i> . <i>J.Reprod. Fert.</i> 112:9-17.
Mons, S. T., et al., "Biological efficiency: How relevant is this concept to beef cows in a mixed livestock seasonal pasture supply context?" <i>Proceedings of the New Zealand Society of Animal Production</i> 54:333, 1994
Monensin." <i>J. Anim. Sci.</i> 55:357-362, 1982
Moran, C., J. F. Quirke, and J. F. Roche. "Puberty in heifers -a review." <i>Animal Reproduction Sci.</i> 18:167, 1989
Morcom, C.B. and Dukelow, W.R. 1980. A research technique for the oviductal insemination of pigs using laparoscopy. <i>Lab. Anim. Sci.</i> 1030-1031.
Morgan, J. B., et al., "National beef tenderness survey." <i>J. Anim. Sci.</i> 69:3274, 1991
Morris, L.H., et al., "Hysteroscopic insemination of small numbers of spermatozoa at the uterotubal junction of preovulatory mares". <i>Journal of Reproduction and Fertility</i> , Vol. 118, pp. 95-100 (2000)
Moseley, W. M., et al., 1982. "Relationship of Growth and Puberty in Beef Heifers Fed
Mount, D. E. "Fibrous and non-fibrous carbohydrate supplementation to ruminants grazing forage from small grain crops." M.S. Thesis. Colorado State University, 2000
Muller, W. and Gautier, F. 1975. Interactions of heteroaromatic compounds with nucleic acids. <i>Euro. J Biochem.</i> 54:358.
Munne, S. 1994. Flow cytometry separation of X and Y spermatozoa could be detrimental to human embryos. <i>Hum. Reprod.</i> 9(5):758
Myers, S. E., "Performance and carcass traits of early-weaned steers receiving either a pasture growing period or a finishing diet at weaning." <i>J. Anim. Sci.</i> 77:311, 1999
Myers, S. E., et al., "Comparison of three weaning ages on cow-calf performance and steer carcass traits." <i>J. Anim. Sci.</i> 77:323, 1999
Myers, S. E., et al., "Production systems comparing early weaning to normal weaning with or without creep feeding for beef steers." <i>J. Anim. Sci.</i> 77:300, 1999
Nix, L. P., L. C. Spitzer, and P. I. Chenoweth. "Serum testosterone concentration, efficiency of estrus detection and libido expression in androgenized beef cows." <i>Therio.</i> 49: 1195, 1998
Nowshari, et al., "Superovulation of Goats with Purified pFSH Supplemented with Defined Amounts of pLH", <i>Theriogenology</i> , Vol. 43, 1995, pp 797-802.
Nowshari, et al., <i>Theriogenology</i> , Vol. 43, 1995, pp 797-802.
NRC. Nutrient requirements for beef cattle. National Academy of Sci. National Research Council, Washington, DC, 1996
Olson, S.E. and Seidel, G.E. Jr., "Reduced Oxygen Tension and EDTA improve Bovine Zygote Development in a Chemically Defined Medium", <i>Journal of Animal Science</i> 78, 2000, pp. 152-157.
Owen, J. B. "The maiden female-a means of increasing meat production." <i>Proc. Symp. on the use of once bred heifers and gilts.</i> 1973
Pace, M.M. and Sullivan, J.J. 1975. Effect of timing of insemination, numbers of spermatozoa and extender components on pregnancy rates in mares inseminated with frozen stallion semen. <i>J Reprod. Fert. Suppl.</i> 23:115-121.
Parent US Application 09/001,394, entitled "Sheath Fluids and Collection Systems for Sex-Specific Cytometer Sorting of Sperm", filed on December 31, 1997, 87 total pages which includes four drawings.
Parrish, J., et al., "Capacitation of Bovine Sperm by Heparin", <i>Technology of Reproduction</i> 38, 1988, pp. 1171-1180.
PCT application, PCT/US99/17165, filed 28 July 1999, entitled "Equine System for Non-Surgical Artificial Insemination".
PCT application, PCT/US98/27909, filed 31 December 1998, entitled "Commercially Practical Sex-Specific Insemination of Mammals".
Peippo, J., et al., "Sex diagnosis of equine preimplantation embryos using the polymerase chain reaction", <i>Theriogenology</i> , Vol. 44 619-627 (1995)
Perry, E.J. 1968. Historical Background In: <i>The Artificial Insemination of Farm Animals</i> , 4 th ed. Edited by R.J. Perry. New Brunswick, Rutgers University Press, pp. 3-12.
Petersen, G.A., et al., "Cow and Calf Performance and Economic Considerations of Early Weaning of Fall-Born

Beef Calves", <i>J. Anim. Sci.</i> , 1987, 64:15, pp 15-22.
Petit, M. "Early Calving in Suckling Herds." In: (Ed.) J.C. Taylor. The early calving of heifers and its impact on beef production. 157, 1975
Pickett GW, et al., "Management of the mare for maximum reproductive efficiency", Bulletin No. 6 Colorado State University, Ft. Collins CO. (1989)
Pickett, B.W., et al., 1976. Factors influencing the fertility of stallion spermatozoa in an A.I. program. Proc. 8 th Internat. Congr. Anim. Reprod. A.I. Krakow, Poland. 4: 1049 - 1052.
Pickett, B.W. and Back, D.G. 1973. Procedures for preparation, collection, evaluation and insemination of stallion semen. C.S.U. Exp. Sta. Anim. Reprod. Lab. Gen. Series Bull. 935.
Pickett, B.W., and Shiner, K.A., "Recent developments in artificial insemination in horses", <i>Livestock Production Science</i> , 40, 1994, pp 31 - 36.
Pickett, B.W., Burwash, L.D., Voss, J.L. and Back, D.G. 1975b. Effect of seminal extenders on equine fertility. <i>J. Anim. Sci.</i> 40:1136-1143.
Pinkel, D., et al, "Flow Cytometric Determination of the Proportions of X- and Y- Chromosome-Bearing Sperm in Samples of Purportedly Separated Bull Sperm", <i>Journal of Animal Science</i> , Vol. 60, No. 5, 1985, pp 1303 - 1307.
Pinkel, D., Gledhill, B.L., Van Dilla, M.A., Stephenson, D. and Watchmaker, G. 1982b. High resolution DNA measurements of mammalian spermatozoa. <i>Cytometry</i> , 3:1-9. (1982b)
Polge, E. J., "Historical Perspective of AI: Commercial Methods of Producing Sex Specific Semen, IVF Procedures", Proceedings of the 16 th Technical Conference on Artificial Insemination & Reproduction, Cambridge, England, 1996, pp. 7-11.
Purvis, H. T. and J. C. Whittier. "Effects of ionophore feeding and anthelmintic administration on age and weight at puberty in spring-born beef heifers." <i>J. Anim. Sci.</i> 74:736-744. 1996
Randel, R. D. "Nutrition and postpartum rebreeding in cattle." <i>J. Anim. Sci.</i> 68:853. 1990
Rath, D., et al., "Low Dose Insemination Technique in the Pig", <i>Boar Semen Preservation IV</i> , 2000, pp. 115-118.
Rath, D., et al., "Production of Piglets Preselected for Sex Following in Vitro Fertilization with X and Y Chromosome-Bearing Spermatozoa Sorted by Flow Cytometry", <i>Theriogenology</i> , 47, 1997, pp 795 - 800.
Reiling, B.A., et al., "Effect of Prenatal Androgenization on Performance, Location, and Carcass and Sensory Traits on Heifers in Single Calf Heifer System", <i>J. Anim. Sci.</i> , 1995, 73: 986, pp 986-992.
Rena, W., et al, "A Novel Nozzle for More Efficient Sperm Orientation to Improve Sorting Efficiency of X and Y Chromosome-Bearing Sperm", <i>Cytometry</i> 33, 1998, pp. 476-481
Rena, W., et al., "Improved Flow Cytometric Sorting of X- and Y- Chromosome Bearing Sperm: Substantial Increase in Yield of Sexed Semen", <i>Molecular Reproduction and Development</i> , 1999, pp 50-56.
Rieger, D., et al, "The Relationship Between the Time of First Cleavage of Fertilized Cattle Oocytes and Their Development to the Blastocyst Stage", <i>Theriogenology</i> , 1999, pp. 190.
Ricar, A. and Ball, A. 1991. Fertility of young cashmere goats after laparoscopic insemination. <i>J. Agr. Sci.</i> 117:271-273.
Roberts, J.R. 1971. In: <i>Veterinary Obstetrics and Genital Diseases</i> . Ithaca, New York. pp. 740-749.
Romita, A. "Some considerations on the beef situation in Italy." (Ed.) J.C. Taylor. The early calving of heifers and its impact on beef production. 23. 1975
Roth, T.L., Wolfe, B.A., Long, J.A., Howard, J. and Wildt, D.E. 1997. Effects of equine chorionic gonadotropin, human chorionic gonadotropin, and laparoscopic artificial insemination on embryo, endocrine, and luteal characteristics in the domestic cat. <i>Bio Reprod.</i> 57:165-171.
Roux, M., J. H. Teissier, J. Bonnemaisre, and R. Dumont. "Early calving heifers versus maiden heifers for beef production from dairy herds. 1." The effects of genotype (Friesian and Charolais x Friesian) and 2 feeding levels in the rearing period on growth and carcass quality. <i>Livestock Prod. Sci.</i> 16:1. 1987
Rowley, H-S., Squires, E.L. and Pickett, B.W. 1990. Effect of insemination volume on embryo recovery in mares. <i>J. Equine Vet. Sci.</i> 10:298-300.
Roy, J. H. B. "Rearing dairy-herd replacements." <i>J. of the Soc. of Dairy Technology</i> 31:73-79. 1978
Rutter, L. M., et al., "Effect of abomasal infusion of propionate on the GnRH-induced luteinizing-hormone release in prepubertal heifers." <i>J. Anim. Sci.</i> 56:1167. 1983
Salamon, S. 1976. <i>Artificial Insemination of Sheep</i> . Chippendale, New South Wales. Publicity Press. p.83-84.
Salisbury, G.W. and VanDemark, N.L. 1961. <i>Physiology of Reproduction and Artificial Insemination of Cattle</i> . San Francisco: Freeman and Company.

SAS. SAS/STAT. "Users Guide (Release 6.03)", SAS Inst. Inc., Cary, NC., 1988. 3 pages
SAS. "The SAS System for Windows." Ver 7.0. Ref 6.12. SAS Inst. Inc., Cary, NC. 2000
Schenk, J. L., T. K. Suh, D. G. Cran, and G. E. Seidel. "Cryopreservation of flow-sorted bovine spermatozoa." <i>Therio. 52:1375. 1999</i>
Schenk, J.L. and Seidel, Jr., G.E., "Imminent Commercialization of Sexed Bovine", Proceedings, The Range Beef Cow Symposium XVI, 1999, pp 89-96.
Schillo, K. K., J. B. Hall, and S. M. Hileman. "Effects of nutrition and season on the onset of puberty in the beef heifer." <i>J. Anim. Sci. 70:3994. 1992</i>
Schmid R.L., et al, "Fertilization with Sexed Equine Spermatozoa Using Intracytoplasmic Sperm Injection and Oviductal Insemination", 7th International Symposium On Equine Reproduction, pp. 139 (Abstract) (1998)
Schnell, T. D., K. E. Bell, J. D. Tatum, R. K. Miller, and G. C. Smith. "Performance, carcass, and palatability traits for cull cows fed high-energy concentrate diets for 0, 14, 28, 42, or 56 days." <i>J. Anim. Sci. 75:1195. 1997</i>
Schoonmaker, J. P., et al., "Effects of age at weaning and implant strategy on growth of steer calves." <i>J. Anim. Sci. (Suppl2) 76:71 (Abstr.). 1998</i>
Seidel, G. E. and L. A. Johnson. "Sexing mammalian sperm -overview." <i>Therio. 52: 1267. 1999</i>
Seidel, G. E., "Insemination of heifers with sexed sperm." <i>Therio. 52:1407. 1999</i>
Seidel, G.E. Jr., "Uterine Horn Insemination of Heifers With Very Low Numbers of Nonfrozen and Sexed Spermatozoa", Atlantic Breeders Cooperative, <i>Theriogenology 48: pp. 1255-1264, (1997)</i>
Seidel, G.E. Jr., Cran, D.G., Herickoff, L.A., Schenk, J.L., Doyle, S.P. and Green, R.D. 1999. Insemination of heifers with sexed frozen or sexed liquid semen. <i>Theriogenology. 51. (in press). abstr.(1999)</i>
Seidel, G.E., Jr., et al, "Artificial Insemination With X-and Y-Bearing Bovine Sperm", Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO; Germplasm and Gamete Physiology Lab, ARS, USDA, Beltsville, MD; Atlantic Breeders Coop, Lancaster, PA; DUO Dairy, Loveland, CO, USA January 1996.
Seidel, G.E., Jr., et al, "Insemination Of Heifers With Very Low Numbers Of Frozen Spermatozoa." , Colorado State University, Fort Collins, Atlantic Breeders Cooperative, Lancaster, PA, DUO Dairy, Loveland, CO, July 1996.
Seidel, Jr., G. E., et al, "Insemination of Holstein Heifers With Very Low Numbers Of Unfrozen Spermatozoa", Colorado State University, Atlantic Breeders Cooperative, (1995)
Seidel, Jr., G.E. et al, "Insemination Of Heifers With Very Low Numbers Of Frozen Spermatozoa", Colorado State University (1996)
Sell, R. S., D. L. Wan, R. D. Little, and T. A. Petry. "Single-calf heifer profitability compared to other north dakota beef production systems." Department of Ag. Eco., North Dakota State University, Ag. Econ. Rpt. 20.
Senger, P.L., Becker, W.C., Davidge, S.T., Hillers, J.K. and Reeves, J.J. 1988. Influence of seasonal insemination on conception rates in dairy cattle. <i>J Anim. Sci. 66:3010-3016.</i>
Shackelford, S. D., M. Koolmarale, and T. L. Wheeler. "Effects of slaughter age on meat tenderness and USDA carcass maturity scores of beef females." <i>J. Anim. Sci. 73:3304. 1995</i>
Shelton, J.N. and Moors, N.W. 1967. The response of the ewe to pregnant mare gonadotropin and to horse anterior pituitary extract. <i>J. Reprod. Fert. 14:175 - 177.</i>
Shilova, A.V., Platov, E.M. and Lebedev, S.G. 1976. The use of human chorionic gonadotrophin for ovulation date regulation in mares. VIIIth Int. Congr. On Anim. Repro. and A.I. 204-208.
Shorthose, W. R. and P. V. Harria. "Effect of animal age on the tenderness of selected beef muscles." <i>J. Food Sci. 55:1-. 1990</i>
Silbermann, M., "Honnones and Cartilage. Cartilage: development, differentiation, and growth." pp. 327-368. Academic Press, Inc. 1983
Simon, M., "The effect of management option on the performance of pregnant feedlot heifers." M.S. Thesis. Kansas State University. 1983
Smith, G. C., B. W. Berry, J. W. Savell, and H. R. Cross. "USDA maturity indexes and palatability of beef rib steaks." <i>J. of Food Quality 11 :1. 1988</i>
Smith, G. C., et al., "Relationship of USDA maturity groups to palatability of cooked beef." <i>J. of Food Sci. 47:1100. 1982</i>
Squires, E., "Simultaneous Analysis of Multiple Sperm Attributes by Flow Cytometry", Diagnostic Techniques and Assisted Reproductive Technology, The Veterinary Clinics of North America, Equine Practice, Vol. 12, No. 1, April 1996, pp127 - 130.

Squires, E.L., Moran, D.M., Farlin, M.E., Jaska, D.J., Keefe, T.J., Meyers, S.A., Figueroa, E., McCue, P.M. and Jochle, W. 1994. Effect of dose of GnRH analogue on ovulation in mares. *Theriogenology*. 41:757-769.

Squires, E.L., "Early Embryonic Loss in Equine Diagnostic Ultrasonography", 1st Ed. pp 157-163 Eds Rantanen & McKinnon. Williams and Wilkins, Baltimore, Maryland (1998)

Squires, E.L., et al, "Cooled and frozen stallion semen", Bulletin No. 9, Colorado State University, Ft. Collins, CO. (1999)

Stallflug, J. N., D. K. Ran, R. D. Randel, and Eo L. Moody. "Plasma estrogens in peri-parturient cow." *Therio* 10:269. 1978

Stevenson, J. S., M. W. Smith, J. R. Jaeger, L. R. Corah, and D. G. Lefever. "Detection of estrus by visual observation and radiotelemetry in peripubertal, estrus-synchronized beefheifers." *J. Anim. Sci.* 74:729. 1996

Story, C. E., R. J. Rasby, R. T. Clark, and C. T. Milton. "Age of calf at weaning of spring-calving beef cows and the effect on cow and calf performance and production economics." *J. Anim. Sci.* 78:1403. 2000

Sullivan, J.J., Parker, W.G. and Larson, L.L. 1973. Duration of estrus and ovulation time in nonlactating mares given human chorionic gonadotropin during three successive estrous periods. *J.A.V.M.A.* 162:895-898.

Swanson, E. W. "Future research on problems of increasing meat production by early calving." *Consn. Eur. Commun., Eur.* 5545.1975. The Early Calving of Heifers and its Impact on Beef Production.

Taljaard, T.L., Terblanche, S.J., Bertschinger, H.J. and Van Vuuren, L.J. 1991. The effect of the laparoscopic insemination technique on the oestrus cycle of the ewe. *J. S Afr. Vet. Assoc.* 62(2):60-61.

Tatum, J. D., G. C. Smith, B. W. Berry, C. E. Murphey, F. L. Williams, and Z. L. Carpenter. "Carcass characteristics, time on feed and cooked beef palatability attributes." *J. Anim. Sci.* 50:833. 1980

Taylor, C.S., Moore, A.J. Thiessen, R.B. and Bailey, C.M., AFRC Animal Breeding Research Organisation, West Mains Road, Edinburg EH9 3JQ, "Efficiency of Food Utilization in Traditional and Sex-Controlled Systems of Beef Production", pp 401-440.

Taylor, S. C. S., A. J. Moore, R. B. Thiessen, and C. M. Bailey. "Efficiency of food utilization in traditional and sex-controlled systems of beef-production." *Animal Production* 40:401. 1983

Tervit, H.R., et al., "Successful Culture In Vitro of Sheep and Cattle Ova", Agricultural Research Council, Unit of Reproduction Physiology and Biochemistry, University of Cambridge. 1972, p. 493-497.

Unruh, J. A. "Effects of endogenous and exogenous growth-promoting compounds on carcass composition, meat quality and meat nutritional-value." *J. Anim. Sci.* 62:1441. 1986

US Application , 09/454,488, entitled "Improved Flow Cytometer Nozzle and Flow Cytometer Sample Handling Methods", filed December 3, 1999.

US Application , 60/238,294, entitled "Hysteroscopic Insemination of Mares" filed October 5, 2000.

US Application , 09/448,643, entitled "Multiple Sexed Embryo Production System for Mammals", filed November 24, 1999.

US Application , 09/511,959 entitled "Methods For Improving Sheath Fluids and Collection Systems For Sex-Specific Cytometer Sorting of Sperm", filed February 23, 2001.

US Application 09/001,394, entitled "Sheath Fluids and Collection Systems for Sex-Specific Cytometer Sorting of Sperm", filed on December 31, 1997, 87 total pages which includes four drawings.

US Application 09/015,454, entitled "System for Improving Yield of Sexed Embryos in Mammals", filed on January 29, 1998, 59 total pages which includes drawings.

US Application 60/211093, entitled "Integrated System for Herd Management Using Sexed Semen", filed June 12, 2000.

US Application entitled "System For Separating Frozen-Thawed Sperm Cells Into X-Chromosome And Y-Chromosome Bearing Populations", filed November 28, 2000.

US Application Serial Number 60/094,720, entitled "System for Low Dose Insemination of Equines", filed July 30, 1998.

US Application Serial Number 60/113,143, entitled "Equine Insemination System", December 18, 1998.

US Application Serial Number 60/203,089, entitled "Detector System for Resolving Small Differences in Photo-generated Signal". filed May 9, 2000.

US Application Serial Number 60/211093, entitled "Integrated System for Herd Management Using Sexed Semen", filed June 12, 2000.

US Application Serial Number 60/224,050., entitled "Integrated System for Herd Management With Terminal-Cross Program Using Sexed Semen", filed August 9, 2000.

USDA "Official United States standards for grades of carcass beef." Agric. Marketing Serv., USDA .Washington,

DC. 1997
Vazquez, J. et al., "Nonsurgical Uterotubal Insemination in the Mare", Proceedings of the 44th Annual Convention of the American Association of Equine Practitioners, Baltimore, Maryland, December 6-9, 1998, Vol. 44, pp 68-69
Vazquez, J., et al., "A.I. in Swine; New Strategy for Deep Insemination with Low Number of Spermatozoa Using a Non-surgical Methodology", 14 th International Congress on Animal Reproduction, Vol. 2, Stockholm, July, 2000, p. 289.
Vazquez, J., et al., "Development of a Non-surgical Deep Intra Uterine Insemination Technique", IV International Conference on Boar Semen Preservation, Maryland, August, 1999, p 35 and photo of display board.
Vazquez, J., et al., "Successful Low-Dose Insemination by a Fiberoptic Endoscope Technique in the Sow", Proceedings Annual Conference of the International Embryo Transfer Society, Netherlands, Theriogenology, Vol. 53, January, 2000, pp. 201.
Vazquez, J., et al., "Hypoosmotic Swelling Test as Predictor of the Membrane Integrity in Boar Spermatozoa", Boar Semen Preservation IV, IVth International Conference on Boar Semen Preservation, Maryland, pp. 263.
Vidament, M., Dupere, A.M., Julienne, P., Evain, A., Noue, P. and Palmer, E. 1997. Equine frozen semen freezeability and fertility field results. Theriogenology. 48:907.
Vincent, B. C., S. D. M. Jones, L. E. Jeremiah, M. A. Price, and J. A. Newman. "Carcass characteristics and meat quality of once-calved heifers." Canadian J. Anim. Sci. 71:311. 1991
Voss, J.L. and Pickett, B.W. 1976. Reproductive management of the broodmare. C.S.U. Exp. Sta. Anim. Reprod. Lab. Gen. Series. Bull. 1-12
Voss, J.L., Pickett, B.W., Burwash, L.D. and Daniels, W.H. 1974. Effect of human chorionic gonadotropin on duration of estrous cycle and fertility of normally cycling, nonlactating mares. J.A.V.M.A. 165:704-706.
Voss, J.L., Squires, E.L., Pickett, B.W., Shideler, R.K. and Eikenberry, D.J. 1982. Effect of number and frequency of inseminations on fertility in mares. J. Reprod. Fertil. Suppl. 32:53-57.
Waggoner, A. W., M. E. Dikeman, L. R. Brethour, and K. E. Kemp. "Performance, carcass, cartilage calcium, sensory and collagen traits of longissimus muscles of open versus 30-month-old heifers that produced one calf." J. Anim. Sci. 68:2380. 1990
Welch G.R., et al., 1994. Fluidic and optical modifications to a FACS IV for flow sorting of X- and Y-chromosome bearing sperm based on DNA. Cytometry 17 (suppl. 7): 74.
Welch, G., et al., "Flow Cytometric Sperm Sorting and PCR to Confirm Separation of X- and Y- Chromosome Bearing Bovine Sperm", Animal Biotechnology, 6 (2), 131-139, 1995, pp 131 - 139.
Wheeler, T. L., L. v. Cundiff, and R. M. Koch. "Effect of marbling degree on beef palatability in Bos- Taurus and Bos-Indicus cattle." J. Anim. Sci. 72:3145. 1994
Wickersham, E. W. and L. H. Schultz. "Influence of age at first breeding on growth, reproduction, and production of well-fed holstein heifers." J. Dairy Sci. 46:544. 1963
Wilson, C.G., Downie, C.R., Hughes, J.P. and Roser, J.F. 1990. Effects of repeated hCG injections on reproductive efficiency in mares. Eq. Vet. Sci. 4:301-308.
Wilson, M.S. 1993. Non-surgical intrauterine artificial insemination in bitches using frozen semen. J.Reprod. Fert. Suppl. 47:307-311.
Woods, J. and Ginther, O.J. 1983. Recent studies related to the collection of multiple embryos in mares. Theriogenology. 19:101 - 108.
Woods, J., Bergfelt, D.R. and Ginther, O.J. 1990. Effects of time of insemination relative to ovulation on pregnancy rate and embryonic-loss rate in mares. Eq. Vet. J. 22(6):410-415.
XP-002103478, File Biosis, one page.

In addition, as to each term used it should be understood that unless its utilization in this application is inconsistent with such interpretation, common dictionary definitions should be understood as incorporated for each term and all definitions, alternative terms, and synonyms such as contained in the Random House Webster's Unabridged Dictionary,

second edition are hereby incorporated by reference. However, as to each of the above, to the extent that such information or statements incorporated by reference might be considered inconsistent with the patenting of this/these invention(s) such statements are expressly not to be considered as made by the applicant(s).

In addition, unless the context requires otherwise, it should be understood that the term "comprise" or variations such as "comprises" or "comprising", are intended to imply the inclusion of a stated element or step or group of elements or steps but not the exclusion of any other element or step or group of elements or steps. Such terms should be interpreted in their most expansive form so as to afford the applicant the broadest coverage legally permissible in countries such as Australia and the like.

Thus, the applicant(s) should be understood to have support to claim at least: i) each of the staining, separation, isolation, insemination, or fertilization procedures as herein disclosed and described, ii) the related methods disclosed and described, iii) similar, equivalent, and even implicit variations of each of these devices and methods, iv) those alternative designs which accomplish each of the functions shown as are disclosed and described, v) those alternative designs and methods which accomplish each of the functions shown as are implicit to accomplish that which is disclosed and described, vi) each feature, component, and step shown as separate and independent inventions, vii) the applications enhanced by the various systems or components disclosed, viii) the resulting products produced by such systems or components, ix) methods and apparatuses substantially as described hereinbefore and with reference to any of the accompanying examples, and x) the various combinations and permutations of each of the elements disclosed.

The claims set forth in this specification are hereby incorporated by reference as part of this description of the invention, and the applicant expressly reserves the right to use all of or a portion of such incorporated content of such claims as additional description to support any of or all of the claims or any element or component thereof, and the applicant further expressly reserves the right to move any portion of or all of the incorporated content of such claims or any element or component thereof from the

description into the claims or vice-versa as necessary to define the subject matter for which protection is sought by this application or by any subsequent continuation, division, or continuation-in-part application thereof, or to obtain any benefit of, reduction in fees pursuant to, or to comply with the patent laws, rules, or regulations of any country or treaty, and such content incorporated by reference shall survive during the entire pendency of this application including any subsequent continuation, division, or continuation-in-part application thereof or any reissue or extension thereon.

VI CLAIMS

I claim:

1. A method of transporting oocytes, comprising the steps of :
 - a. collecting oocytes from a female mammal;
 - b. entraining oocytes in a fertilization medium;
 - c. transferring oocytes in said fertilization medium into a straw;
 - d. sealing said straw containing said oocytes;
 - e. transferring at least one straw inside of an incubation element;
 - f. establishing incubation conditions within said incubation element;
 - g. sealing said incubation element;
 - h. transporting said oocytes from said female mammal within said straw inside of said incubation element.
2. A method of transporting oocytes as described in claim 1, wherein said female mammal is selected from the group consisting of primates, humans, bovids, ovids, equids, swine, and dolphins.
3. A method of transporting oocytes as described in claim 1, wherein said fertilization medium comprises modified Tyrode's medium supplemented with 0.6 percent bovine serum albumin, 20µg heparin per milliliter of Tyrode's medium, and a concentration of 5 milli-molar caffeine.
4. A method of transporting oocytes as described in claim 1, wherein between about 10 and about 15 of said oocytes are contained within about 50 micro-liters of said fertilization medium.
5. A method of transporting oocytes as described in claim 1, wherein said straw has heat sealable aperture elements.

6. A method of transporting oocytes as described in claim 5, wherein said straw has a interior volume of about 0.25 milliliters.
7. A method of transporting oocytes as described in claim 1, wherein said incubation element has sealable aperture elements.
8. A method of transporting oocytes as described in claim 7, wherein said incubation element comprises a glass tube.
9. A method of transporting oocytes as described in claim 1, wherein said incubation conditions comprise an atmosphere of 5 percent carbon dioxide in air and a temperature of 39 degrees Centigrade within said incubation element.
10. A method of transporting oocytes as described in claim 1, further comprising the step of transferring sperm cells to said straw containing said oocytes in said fertilization medium.
11. A method of transporting oocytes as described in claim 10, wherein said step of transferring sperm cells to said straw containing said oocytes comprises establishing a concentration of sperm cells in said fertilization medium of about 1 million to about 2 million per milliliter of fertilization medium.
12. A method of transporting oocytes as described in claim 1, further comprising the step of separating spermatozoa into enriched X-chromosome bearing and Y-chromosome populations.
13. A method of transporting oocytes as described in claim 12, further comprising the step of transferring separated sperm cells to said straw containing oocytes.
14. A method of transporting oocytes as described in claim 13, wherein said step of transferring separated sperm cells to said straw containing oocytes comprises establishing a concentration of said separated sperm cells in said fertilization

medium of about 1 million to about 2 million per milliliter of fertilization medium.

15. A method of transporting oocytes as described in claims 10, 11, 12, 13, or 14 further comprising the step of transferring said straw containing said oocytes in said concentration of said sperm cells to said incubation element.
16. A method of transporting oocytes as described in claim 15, further comprising the step of establishing fertilization conditions within said incubation element.
17. A method of transporting oocytes as described in claim 16, wherein said step of establishing fertilization conditions within said incubation element comprises an atmosphere of 5 percent carbon dioxide in air at a temperature between about 37 degrees Centigrade and about 41 degrees Centigrade for a duration of about 18 hours to about 20 hours.
18. A method of transporting oocytes as described in claim 16, further comprising the step of transporting said oocytes in said fertilization conditions.
19. A method of transporting oocytes as described in claim 18, further comprising the step of fertilizing at least some of said oocytes during transport.
20. A method of fertilizing oocytes, comprising the steps of:
 - a. collecting sperm cells from a male mammal;
 - b. transferring said sperm cells to a straw containing a plurality of oocytes in fertilization medium, wherein said oocytes are from a female mammal of the same species as said male mammal;
 - c. sealing said straw containing said sperm cells and said oocytes;
 - d. transferring said straw containing said sperm cells and said oocytes to the inside of an incubation element;
 - e. establishing incubation conditions within said incubation element;
 - f. sealing said incubation element; and

- g. fertilizing at least one oocyte in said straw.
21. A method of transporting oocytes as described in claim 20, wherein said female mammal is selected from the group consisting of primates, humans, bovids, ovids, equids, swine, and dolphins.
22. A method of transporting oocytes as described in claim 20, wherein said fertilization medium comprises modified Tyrode's medium supplemented with 0.6 percent bovine serum albumin, 20 μ g heparin per milliliter of Tyrode's medium, and a concentration of 5 milli-molar caffeine.
23. A method of transporting oocytes as described in claim 20, wherein between about 10 and about 15 of said oocytes are contained within about 50 micro-liters of said fertilization medium.
24. A method of transporting oocytes as described in claim 20, wherein said straw has heat sealable aperture elements.
25. A method of transporting oocytes as described in claim 24, wherein said straw has a interior volume of about 0.25 milliliters.
26. A method of transporting oocytes as described in claim 20, wherein said incubation element has sealable aperture elements.
27. A method of transporting oocytes as described in claim 26, wherein said incubation element comprises a glass tube.
28. A method of transporting oocytes as described in claim 20, wherein said incubation conditions comprise an atmosphere of 5 percent carbon dioxide in air and a temperature of 39 degrees Centigrade within said incubation element.

29. A method of transporting oocytes as described in claim 20, further comprising the step of transferring said sperm cells to said straw containing said oocytes in said fertilization medium.
30. A method of transporting oocytes as described in claim 29, wherein said step of transferring sperm cells to said straw containing said oocytes comprises establishing a concentration of sperm cells in said fertilization medium of about 1 million to about 2 million per milliliter of fertilization medium.
31. A method of transporting oocytes as described in claim 21, further comprising the step of separating said sperm cells into enriched X-chromosome bearing and Y-chromosome populations.
32. A method of transporting oocytes as described in claim 31, further comprising the step of transferring separated sperm cells to said straw containing oocytes.
33. A method of transporting oocytes as described in claim 32, wherein said step of transferring separated sperm cells to said straw containing oocytes comprises establishing a concentration of said separated sperm cells in said fertilization medium of about 1 million to about 2 million per milliliter of fertilization medium.
34. A method of transporting oocytes as described in claims 29, 30, 31, 32, or 33 further comprising the step of transferring said straw containing said oocytes in said concentration of said sperm cells to said incubation element.
35. A method of transporting oocytes as described in claim 34, further comprising the step of establishing fertilization conditions within said incubation element.
36. A method of transporting oocytes as described in claim 35, wherein said step of establishing fertilization conditions within said incubation element comprises an atmosphere of 5 percent carbon dioxide in air at a temperature between about 37

degrees Centigrade and about 41 degrees Centigrade for a duration of about 18 hours to about 20 hours.

37. A method of transporting oocytes as described in claim 35, further comprising the step of transporting said oocytes in said fertilization conditions.
38. A method of transporting oocytes as described in claim 37, further comprising the step of fertilizing at least some of said oocytes during transport.
39. A method of transporting oocytes as described in claim 37, further comprising the step of removing fertilized oocytes from said straw.
40. A method of transporting oocytes as described in claim 37, further comprising the step of implanting said fertilized oocytes into a female mammal.
41. A method of transporting oocytes as described in claim 37, wherein said step of implanting said fertilized oocytes into a female mammal comprises implanting said fertilized oocytes into the same species of said female mammal as said male mammal.
42. A method of transporting oocytes as described in claim 37, wherein said step of implanting said fertilized oocytes into female mammal comprises implanting fertilized oocytes into a different species of said female mammal than said male mammal.
43. A mammal having predetermined sex produced according to claims 35, 37, 38, 39, 40, 41, or 42.
44. A portable incubation system, comprising:
 - a. a straw configured to contain fertilization medium into which a plurality of oocytes from a female mammal and sperm cells from a male mammal are transferred;

- b. an incubator element configured to encapsulate said straw containing fertilization medium into said oocytes from said female and said sperm cells from said male mammal have been transferred; and
 - c. an incubation environment maintained within said incubator element, comprising:
 - i. an incubation atmosphere of about 5 per cent carbon dioxide in air, and
 - ii. a temperature between about 37 degrees Centigrade and about 41 degrees Centigrade.
45. A portable incubation system as described in claim 44, wherein said mammal is selected from the group consisting of primates, humans, bovids, ovids, equids, swine, and dolphins.
46. A portable incubation system as described in claim 44, wherein said fertilization medium comprises Tyrode's medium supplemented with 0.6 percent bovine serum albumin, 20 µg heparin per milliliter of fertilization medium, and a concentration of 5 millimolar caffeine.
47. A portable incubation system as described in claim 44, wherein said plurality of oocytes from a female mammal comprises about 10 oocytes to about 15 oocytes per 50 microliters of fertilization medium.
48. A portable incubation system as described in claim 44, wherein said sperm cells from said male mammal have a concentration of about 1 million to about 2million per milliliter of said fertilization medium.
49. A portable incubation system as described in claim 44, wherein said sperm cells comprise sperm cells separated on the basis of bearing an X-chromosome or bearing a Y-chromosome.

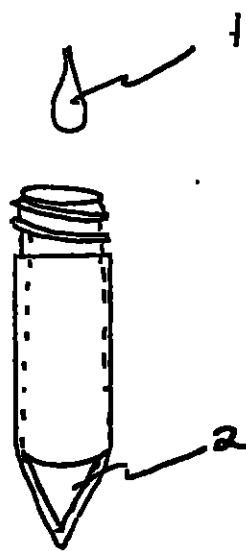
50. A portable incubation system as described in claim 44, wherein said incubation element comprises glass tube.
51. A portable incubation system as described in claim 44, wherein said incubation environment is maintained between about 18 hours and about 20 hours.
52. A portable incubation system as described in claim 44, further comprising a transportation element to move said portable incubation system the distance between two locations.
53. A portable incubation system as described in claim 52, wherein fertilization of said oocytes from a female with said sperm cells from said male mammal within said fertilization medium contained by said straw occurs during transport of said portable incubator system.
54. A portable incubation system as described in claim 53, wherein said two locations comprise a sperm cell separation facility having a first location and a female mammal into which fertilized oocytes are implanted having a second location.
55. A portable incubation system as described in claim 44, wherein said a straw configured to contain fertilization medium into which a plurality of oocytes from a female mammal and sperm cells from a male mammal are transferred has a volume of 0.25 milliliters.
56. A method of in vitro fertilization of oocytes, comprising the steps of :
- a. collecting oocytes from a female mammal;
 - b. entraining a plurality of oocytes in a fertilization medium containing both essential and non-essential amino acids;
 - c. inseminating said oocytes with sperm cells collected from a male mammal of the same species as said female mammal;
 - d. establishing inseminated oocytes in incubation conditions within an incubation element; and

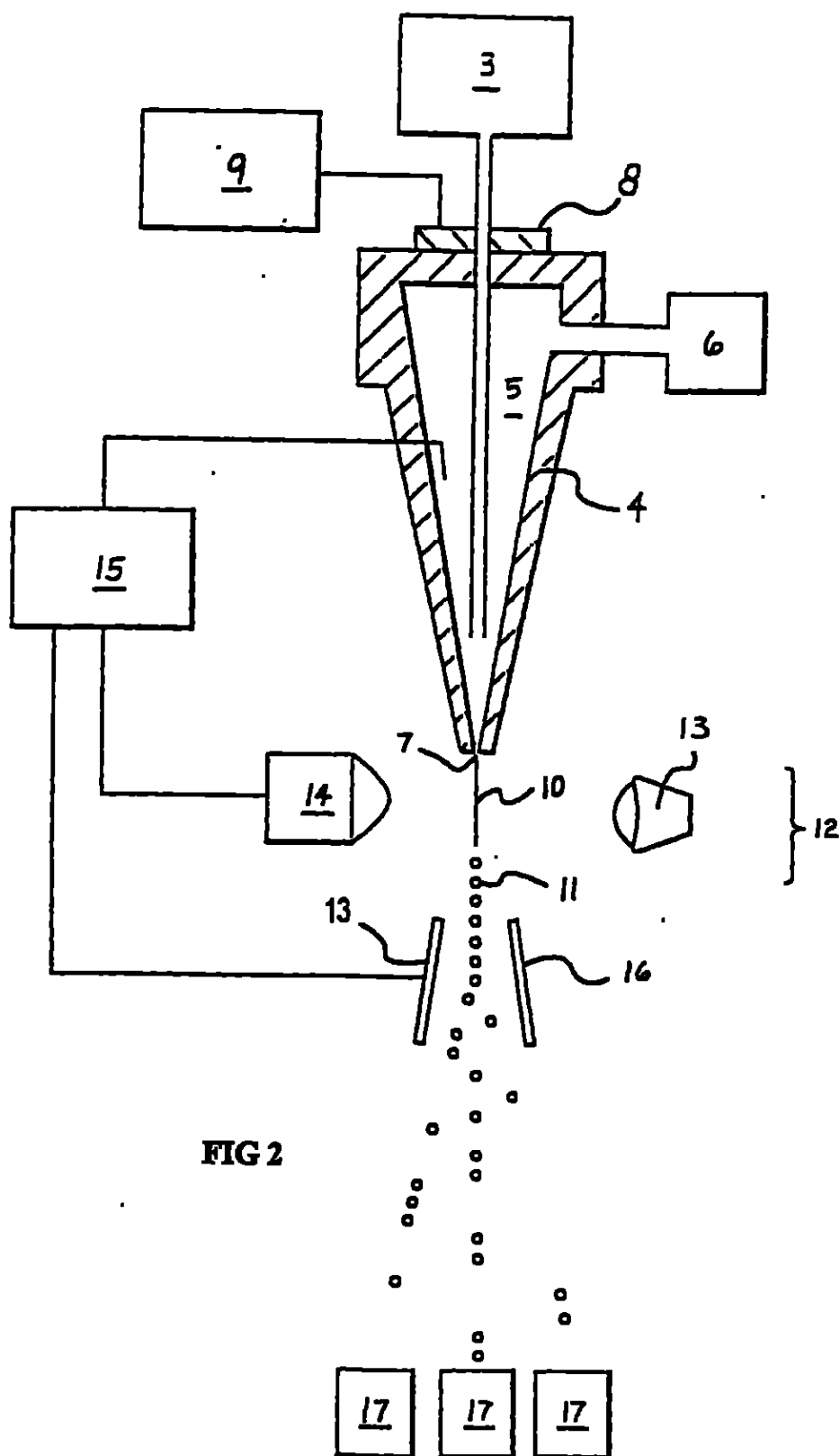
e. fertilizing at least one of said oocytes.

57. A method of in vitro fertilization of oocytes as described in claim 56, wherein said fertilization medium is selected from the group consisting of chemically defined medium, SOF, or TALP supplemented with Eagles Medium.
58. A method of in vitro fertilization of oocytes as described in claim 56, wherein said female mammal is selected from the group consisting of primates, humans, bovids, ovids, equids, swine, and dolphins.
59. A method of in vitro fertilization of oocytes as described in claim 56, wherein said non-essential amino acids have a concentration in said fertilization medium are further supplemented with essential amino acids.
60. A method of in vitro fertilization of oocytes as described in claim 56, entraining a plurality of oocytes in a fertilization medium containing both essential and non-essential amino acids comprises entraining about 10 oocytes to about 15 oocytes in about 50 microliters of fertilization medium.
61. A method of in vitro fertilization of oocytes as described in claim 56, wherein said step of establishing oocytes in incubation conditions within said incubation element comprises an atmosphere of 5 percent carbon dioxide in air at a temperature between about 37 degrees Centigrade and about 41 degrees Centigrade for a duration of about 18 hours to about 20 hours.
62. A method of in vitro fertilization of oocytes as described in claim 56, wherein said step of inseminating said oocytes with sperm cells collected from a male mammal of the same species as said female mammal comprises establishing a concentration of said separated sperm cells in said fertilization medium of about 1 million to about 2 million per milliliter of fertilization medium.

63. A method of in vitro fertilization of oocytes as described in claim 56, wherein said sperm cells are separated on the basis of bearing an X-chromosome or bearing a Y-chromosome.

FIG 1





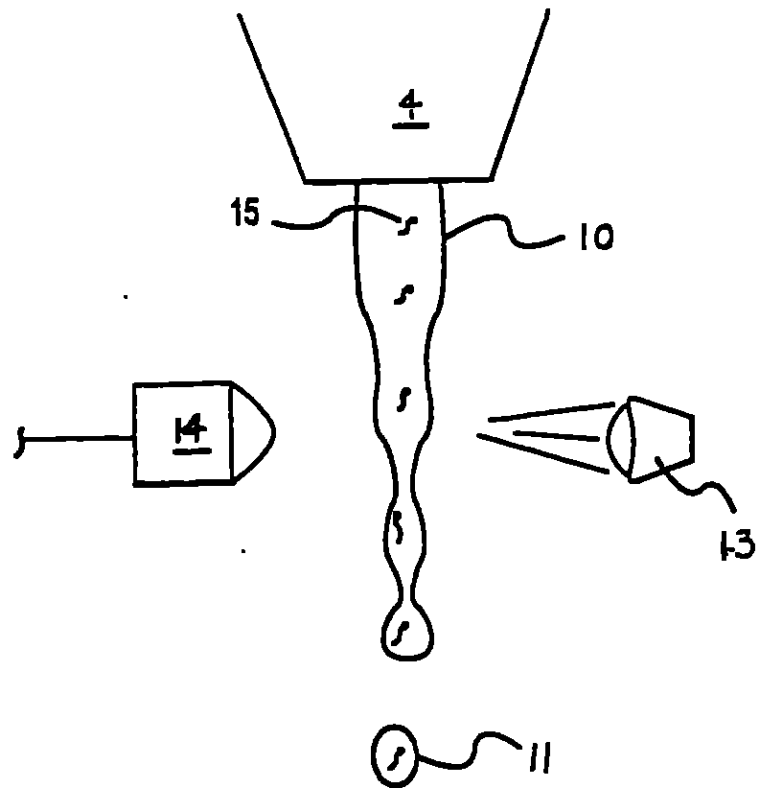
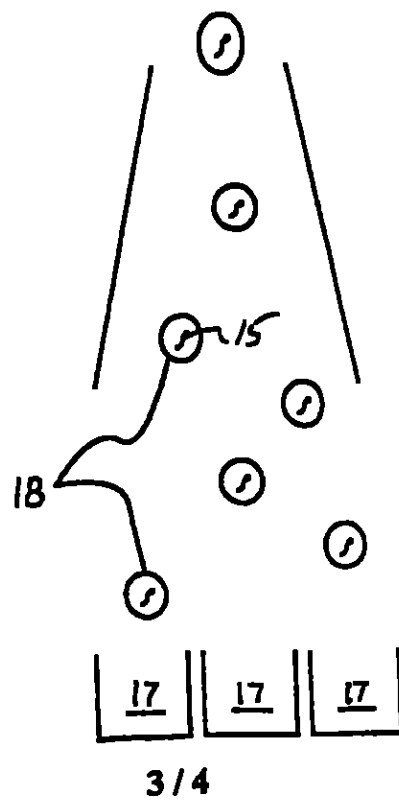


FIG 3



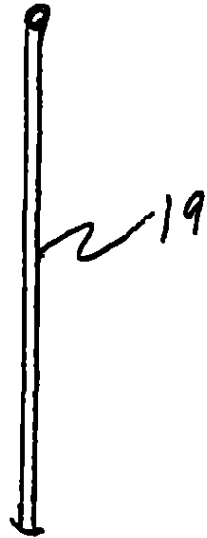
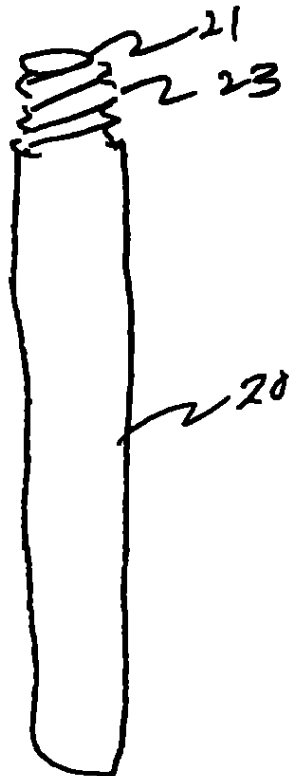


FIG 4



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/45237

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A01N 1/00
US CL : 435/2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
USPATFULL, MEDLINE, BIOSIS, WPIDS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,119,465 A (MULLENS et al.) 19 september 2000.	1-19
A,P	US 6,238,920 B1 (NAGAI et al.) 29 May 2001.	1-19

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"Z" document member of the same patent family

Date of the actual completion of the international search

19 April 2002 (19.04.2002)

Date of mailing of the international search report

14 MAY 2002

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20501

Facsimile No. (703)305-3230

Authorized officer

Dorothy Lawrence For

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/45237

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-19

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/45237

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-19, drawn to a first method, a method of transporting oocytes.

Group II, claims 20-42, drawn to a second method, a method of fertilizing oocytes.

Group III, claim 43, drawn to a mammal of a preselected gender.

Group IV, claims 44-55, drawn to an incubation system.

Group V, claims 56-63 drawn to a third method, a method of in vitro fertilizing of oocytes. The inventions listed as Groups I-V do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I, II and V are directed to three different methods because they have distinct steps. Multiple methods are not permitted under PCT Rule 13.1.

Group III does not relate to a single general inventive concept under PCT Rule 13.2 because sex selection of mammals is well known in the prior art and further, the animal, per se, cannot be distinguished from an animal whose gender was not predetermined.

Group IV is not related to a single general inventive concept under PCT Rule 13.2 because the incubation system of Group IV where the temperature and CO₂ levels are stipulated is not required to be used in the methods of Groups I, II or V where the methods of claims 1, 20 or 56 do not stipulate any temperature or gas concentration.

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference
(if desired) (12 characters maximum) XY-IVF-PCT

Box No. I TITLE OF INVENTION System For In-vitro Fertilization With Spermatozoa Separated Into X-Chromosome And Y-Chromosome Bearing Populations	
Box No. II APPLICANT ☐ This person is also inventor	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) XY, INC. 1108 North Lemay Avenue Fort Collins, Colorado 80524 United States of America	
Telephone No. (970) 493-3113	
Facsimile No. (970) 493-3114	
Teleprinter No.	
Applicant's registration No. with the Office	
State (that is, country) of nationality: US	State (that is, country) of residence: US
This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.) COLORADO STATE UNIVERSITY through its agent Colorado State University Research Foundation 601 South Howes, Room 410 Fort Collins, Colorado 80522 United States of America	
This person is: ☒ applicant only ☐ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.)	
Applicant's registration No. with the Office	
State (that is, country) of nationality: US	State (that is, country) of residence: US
This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.	
Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE	
The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) MILES, Craig R. Santangelo Law Offices, p.C. 125 South Howes, Third Floor Fort Collins, Colorado 80521 United States of America	
Telephone No. (970) 224-3100	
Facsimile No. (970) 224-3175	
Teleprinter No.	
Agent's registration No. with the Office 45,954	
☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.	

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S) <i>If none of the following sub-boxes is used, this sheet should not be included in the request.</i>	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</i> SEIDEL, George E. c/o XY, Inc. 1108 North Lemay Avenue Fort Collins, Colorado 80524 United States of America	This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State (that is, country) of nationality: US	State (that is, country) of residence: US
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</i> SUH, Tae Kwang c/o XY, Inc. 1108 North Lemay Avenue Fort Collins, Colorado 80524 United States of America	This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State (that is, country) of nationality: KR	State (that is, country) of residence: US
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</i> LU, Kehaun c/o XY, Inc. 1108 North Lemay Avenue Fort Collins, Colorado 80524 United States of America	This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State (that is, country) of nationality: UK	State (that is, country) of residence: UK
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</i> 	This person is: ☐ applicant only ☐ applicant and inventor ☐ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State (that is, country) of nationality:	State (that is, country) of residence:
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.	

Box No. V DESIGNATION OF STATES *Mark the applicable check-boxes below: at least one must be marked.*

The following designations are hereby made under Rule 4.9(a):

Regional Patent

☒ AP

ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

(5)☒ EA

Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

(2)☒ EP

European Patent: AT Austria, BE Belgium, CH & LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, TR Turkey, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ OA

OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (If other kind of protection or treatment desired, specify on dotted line)

National Patent (If other kind of protection or treatment desired, specify on dotted line):

☒ AE United Arab Emirates	☒ GH Ghana	(4) ☒ MX Mexico
☒ AG Antigua and Barbuda	☒ GM Gambia	☒ MZ Mozambique
☒ AL Albania	☒ HR Croatia	☒ NO Norway
☒ AM Armenia	☒ HU Hungary	☒ NZ New Zealand
☒ AT Austria and Utility Model	☒ ID Indonesia	☒ PL Poland
(5) ☒ AU Australia	☒ IL Israel	☒ PT Portugal
☒ AZ Azerbaijan	☒ IN India	☒ RO Romania
☒ BA Bosnia and Herzegovina	☒ IS Iceland	☒ RU Russian Federation
☒ BB Barbados	☒ JP Japan	
☒ BG Bulgaria	☒ KE Kenya	☒ SD Sudan
☒ BR Brazil	☒ KG Kyrgyzstan	☒ SE Sweden
☒ BY Belarus	☒ KP Democratic People's Republic of Korea	☒ SG Singapore
☒ BZ Belize	☒ KR Republic of Korea	☒ SI Slovenia
(3) ☒ CA Canada	☒ KZ Kazakhstan	☒ SK Slovakia & Utility Model
☒ CH & LI Switzerland and Liechtenstein	☒ LC Saint Lucia	☒ SL Sierra Leone
☒ CN China	☒ LK Sri Lanka	☒ TJ Tajikistan
☒ CO Colombia	☒ LR Liberia	☒ TM Turkmenistan
☒ CR Costa Rica	☒ LS Lesotho	☒ TR Turkey
☒ CU Cuba	☒ LT Lithuania	☒ TT Trinidad and Tobago
☒ CZ Czech Republic & Utility Model	☒ LU Luxembourg	
☒ DE Germany & Utility Model	☒ LV Latvia	☒ TZ United Republic of Tanzania
☒ DK Denmark & Utility Model	☒ MA Morocco	☒ UA Ukraine
☒ DM Dominica	☒ MD Republic of Moldova	☒ UG Uganda
☒ DZ Algeria		(1) ☒ US United States of America
☒ EC Ecuador	☒ MG Madagascar	
☒ EE Estonia and Utility Model	☒ MK The former Yugoslav Republic of Macedonia	☒ UZ Uzbekistan
☒ ES Spain	☒ MN Mongolia	☒ VN Viet Nam
☒ FI Finland and Utility Model	☒ MW Malawi	☒ YU Yugoslavia
☒ GB United Kingdom		☒ ZA South Africa
☒ GD Grenada		☒ ZW Zimbabwe
☒ GE Georgia		

Check-boxes below reserved for designating States which have become party to the PCT after issuance of this sheet:

☒ All possible states & all possible countries that have become members of the PCT since issuance of this form

☒ GQ Equatorial Guinea

☒ RH Philippines

☒ OM Oman

☒ ZM Zambia

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)

Supplemental Box
If the Supplemental Box is not used, this sheet should not be included in the request.

1. *If, in any of the Boxes, except Boxes Nos. I-III (i) to (iv) for which a special continuation box is provided, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ..." (Indicate the number of the Box) and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:*
 - (i) *If more than two persons are to be indicated as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;*
 - (ii) *If, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;*
 - (iii) *If, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;*
 - (iv) *If, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*
 - (v) *If, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part": in such case, write "Continuation of Box No. V" and the name of each State involved (for OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*
 - (vi) *If, in Box No. VI, there are more than five earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.*
2. *If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.*

CONTINUATION OF BOX VI:

This application claims priority of US patent application no. 60/253,785, filed on 29 November 2000 (29.11.00) and priority of US patent application no. 60/253, 737, filed on 29 November 2000 (29.11.00).

Box No. VI PRIORITY CLAIM				
The priority of the following earlier application(s) is hereby claimed:				
Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country	regional application: regional Office	international application: receiving Office
item (1) (29.11.00) 29 November 2000	60/253,785	US		
item (2) (29.11.00) 29 November 2000	60/253,787	US		
item (3)				
item (4)				
item (5)				
☒ Further priority claims are indicated in the Supplemental Box.				
The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office) identified above as:				
☒ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ item (4) ☐ item (5) ☐ other, see Supplemental Box				
* Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(ii)):				
Box No. VII INTERNATIONAL SEARCHING AUTHORITY				
Choice of International Searching Authority (ISA) (if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):				
ISA / ...US				
Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):				
Date (day/month/year)		Number	Country (or regional Office)	
Box No. VIII DECLARATIONS				
The following declarations are contained in Boxes Nos. VIII (i) to (v) (mark the applicable check-boxes below and indicate in the right column the number of each type of declaration):				Number of declarations
☐	Box No. VIII (i)	Declaration as to the identity of the inventor	:	
☐	Box No. VIII (ii)	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	:	
☐	Box No. VIII (iii)	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	:	
☒	Box No. VIII (iv)	Declaration of inventorship (only for the purposes of the designation of the United States of America)	:	1
☐	Box No. VIII (v)	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	:	

Box No. VIII (iv) **DECLARATION: INVENTORSHIP** (only for the purposes of the designation of the United States of America)
The declaration must conform to the following standardized wording provided for in Section 114: see Notes to Boxes Nos. VII, VII (i) to (iv)
in general and the specific Notes to Box No. VIII (iv). If this box is not used, the sheet should not be included in the request.

**Declaration of Inventorship (Rules 4.17(iv) and 51b.1(a)(vi))
for the purposes of the designation of the United States of America:**

I hereby declare that I believe I am the original, first and sole (if only one inventor is listed below) or joint (if more than one inventor is listed below) inventor of the subject matter which is claimed and for which a patent is sought.

This declaration is directed to the international application of which is forms a part (if filing declaration with application).

This declaration is directed to international application No. PCT/US01/45237 (if furnishing declaration pursuant to Rule 26ter).

I hereby declare that my residence, mailing address, and citizenship are as stated next to my name.

I hereby state that I have reviewed and understand the contents of the above-identified international application, including the claims of said application. I have identified in the request of said application, in compliance with PCT Rule 4.10, any claims to foreign priority, and I have identified below, under the heading "Prior Applications," by application number, country or Member of the World Trade Organization, day, month and year of filing, any application for a patent or inventor's certificate filed in a country other than the United States of America, including any PCT international application designating at least one country other than the United States of America, having a filing date before that of the application on which foreign priority is claimed.

Prior Applications: US 60/253,785, US 60/253,787, both filed 29 November 2000

I hereby acknowledge the duty to disclose information that is known by me to be material to patentability as defined by 37 C.F.R. § 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the PCT international filing date of the continuation-in-part application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name: George E. Seidel

Residence: 3101 Arrowhead Road, Colorado 80535 USA
(city and either US state, if applicable, or country)

Mailing Address: 3101 Arrowhead Road
Colorado 80535 USA

Citizenship: United States of America

Inventor's Signature: George E. Seidel
(if not contained in the request, or if declaration is corrected or added under Rule 26ter after the filing of the international application. The signature must be that of the inventor, not that of the agent)

Date: December 5, 2001
(of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26ter after the filing of the international application)

Name: Tae Kwang Suh

Residence: 2044 Glenmoor Drive, Fort Collins, Colorado 80521 USA
(city and either US state, if applicable, or country)

Mailing Address: 2044 Glenmoor Drive
Fort Collins, Colorado 80521 USA

Citizenship: South Korea

Inventor's Signature: Tae Kwang Suh
(if not contained in the request, or if declaration is corrected or added under Rule 26ter after the filing of the international application. The signature must be that of the inventor, not that of the agent)

Date: December 5, 2001
(of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26ter after the filing of the international application)

☒ This declaration is continued on the following sheet, "Continuation of Box No. VIII (iv)".

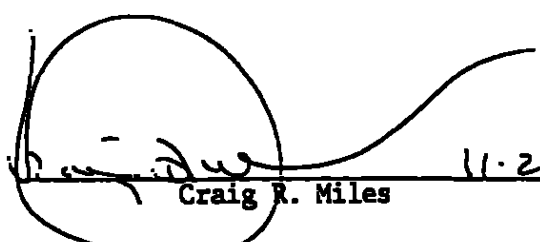
Continuation of Sheet No. VII as (v) DECLARATION
(This space is for the continuation of Sheet No. VII (a) as (v) to provide all the information, including in the case where more than two inventors are to be named in sheet No. VII (b), in such cases with "Continuation of Sheet No. VII -" (insert the true number of the sheet and furnish the information in the same manner as required for the purpose of the sheet in which the space was transferred. If additional space is needed in respect of two or more declarations, a separate continuation sheet must be used for each such declaration. If this sheet is not used, this sheet should not be included in the request.)

Name: Kebuan Li
Residence: 14 Burrough Field
 Ipsington, Cambridge CB4 9NW
 United Kingdom
Mailing Address: 14 Burrough Field
 Ipsington, Cambridge CB4 9NW
 United Kingdom
Citizenship: United Kingdom

Inventor's Signature: 

Date: November , 2001

Feb 11, 2002

Box No. IX CHECK LIST: LANGUAGE OF FILING																											
This international application contains: (a) the following number of sheets in paper form: request (including declaration sheets) : 3 description (excluding sequence listing part) : 29 claims : 10 abstract : 1 drawings : 1 Sub-total number of sheets : 52 sequence listing part of description (actual number of sheets if filed in paper form, whether or not also filed in computer readable form: see (b) below) : --- Total number of sheets : 52 (b) sequence listing part of description filed in computer readable form (i) ☐ only (under Section 801(a)(i)) (ii) ☐ in addition to being filed in paper form (under Section 801(a)(ii)) Type and number of carriers (diskette, CD-ROM, CD-R or other) on which the sequence listing part is contained (additional copies to be indicated under item 9(ii), in right column):	This international application is accompanied by the following items: (mark the applicable check-boxes below and indicate in right column the number of each item): <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 80%;">1. ☒ fee calculation sheet</td> <td style="width: 20%; text-align: right;">: 1</td> </tr> <tr> <td>2. ☒ original separate power of attorney (2) and Power of Agent (2)</td> <td style="text-align: right;">: 4</td> </tr> <tr> <td>3. ☐ original general power of attorney</td> <td style="text-align: right;">:</td> </tr> <tr> <td>4. ☐ copy of general power of attorney; reference number, if any:</td> <td style="text-align: right;">:</td> </tr> <tr> <td>5. ☐ statement explaining lack of signature</td> <td style="text-align: right;">:</td> </tr> <tr> <td>6. ☐ priority document(s) identified in Box No. VI as item(s):</td> <td style="text-align: right;">:</td> </tr> <tr> <td>7. ☐ translation of international application into (language):</td> <td style="text-align: right;">:</td> </tr> <tr> <td>8. ☐ separate indications concerning deposited microorganism or other biological material</td> <td style="text-align: right;">:</td> </tr> <tr> <td>9. ☐ sequence listing in computer readable form (indicate also type and number of carriers (diskette, CD-ROM, CD-R or other))</td> <td style="text-align: right;">:</td> </tr> <tr> <td style="padding-left: 20px;">(i) ☐ copy submitted for the purposes of international search under Rule 13ter only (and not as part of the international application)</td> <td style="text-align: right;">:</td> </tr> <tr> <td style="padding-left: 20px;">(ii) ☐ (only where check-box (b)(i) or (b)(ii) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Rule 13ter</td> <td style="text-align: right;">:</td> </tr> <tr> <td style="padding-left: 20px;">(iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listing part mentioned in left column</td> <td style="text-align: right;">:</td> </tr> <tr> <td>10. ☒ other (specify): USPO Letter of Transmittal; Mailing Certificates; Letter of Transmittal</td> <td style="text-align: right;">:</td> </tr> </table>	1. ☒ fee calculation sheet	: 1	2. ☒ original separate power of attorney (2) and Power of Agent (2)	: 4	3. ☐ original general power of attorney	:	4. ☐ copy of general power of attorney; reference number, if any:	:	5. ☐ statement explaining lack of signature	:	6. ☐ priority document(s) identified in Box No. VI as item(s):	:	7. ☐ translation of international application into (language):	:	8. ☐ separate indications concerning deposited microorganism or other biological material	:	9. ☐ sequence listing in computer readable form (indicate also type and number of carriers (diskette, CD-ROM, CD-R or other))	:	(i) ☐ copy submitted for the purposes of international search under Rule 13ter only (and not as part of the international application)	:	(ii) ☐ (only where check-box (b)(i) or (b)(ii) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Rule 13ter	:	(iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listing part mentioned in left column	:	10. ☒ other (specify): USPO Letter of Transmittal; Mailing Certificates; Letter of Transmittal	:
1. ☒ fee calculation sheet	: 1																										
2. ☒ original separate power of attorney (2) and Power of Agent (2)	: 4																										
3. ☐ original general power of attorney	:																										
4. ☐ copy of general power of attorney; reference number, if any:	:																										
5. ☐ statement explaining lack of signature	:																										
6. ☐ priority document(s) identified in Box No. VI as item(s):	:																										
7. ☐ translation of international application into (language):	:																										
8. ☐ separate indications concerning deposited microorganism or other biological material	:																										
9. ☐ sequence listing in computer readable form (indicate also type and number of carriers (diskette, CD-ROM, CD-R or other))	:																										
(i) ☐ copy submitted for the purposes of international search under Rule 13ter only (and not as part of the international application)	:																										
(ii) ☐ (only where check-box (b)(i) or (b)(ii) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Rule 13ter	:																										
(iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listing part mentioned in left column	:																										
10. ☒ other (specify): USPO Letter of Transmittal; Mailing Certificates; Letter of Transmittal	:																										
Figure of the drawings which should accompany the abstract Fig. 4	Language of filing of the international application: English																										
Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE <i>Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).</i>																											
 Craig R. Miles 11.29.01																											
For receiving Office use only																											
1. Date of actual receipt of the purported international application: 3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application: 4. Date of timely receipt of the required corrections under PCT Article 11(2): 5. International Searching Authority (if two or more are competent): ISA /	2. Drawings: ☐ received: ☐ not received: 6. ☐ Transmittal of search copy delayed until search fee is paid																										
For International Bureau use only																											
Date of receipt of the record copy by the International Bureau:																											

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
CRAIG R. MILES
SANTANGELO LAW OFFICES, P.C.
125 SOUTH HOWES, THIRD FLOOR
FORT COLLINS, COLORADO 80521

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

(PCT Rule 44.1)

Date of Mailing (day/month/year) 14 MAY 2002	
Applicant's or agent's file reference XY-IVF-PCT	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/US01/43237	International filing date (day/month/year) 29 November 2001 (29.11.2001)
Applicant XY, INC.	

1. ☒ The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

Where? Directly to the International Bureau of WIPO, 34, chemin des Colombettes
1211 Geneva 20, Switzerland, Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Reminders

Shortly after 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90 bis.1 and 90 bis.3, respectively, before the completion of the technical preparations for international publication.

Within 19 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later); otherwise the applicant must, within 30 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 19 months.

See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the PCT Applicant's Guide, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the ISA/US Commissioner for Patents Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer <i>Dorothea Lawrence</i> Sandra Soucier Telephone No. (703) 308-0196
---	--

Form PCT/ISA/220 (April 2002) (See notes on accompanying sheet)

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
CRAIG R. MILES
SANTANGELO LAW OFFICES, P.C.
125 SOUTH HOWES, THIRD FLOOR
FORT COLLINS, COLORADO 80521

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

(PCT Rule 44.1)

Date of Mailing
(day/month/year)

14 MAY 2002

Applicant's or agent's file reference
XY-IVF-PCT

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.
PCT/US01/45237

International filing date
(day/month/year)

29 November 2001 (29.11.2001)

Applicant
XY, INC.

1. ☒ The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

Where? Directly to the International Bureau of WIPO, 34, chemin des Colombettes
1211 Geneva 20, Switzerland, Facsimile No.: (41-22) 740.14.33

For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest: the applicant will be notified as soon as a decision is made.

4. Reminders

Shortly after 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90 bis.1 and 90 bis.3, respectively, before the completion of the technical preparations for international publication.

Within 19 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later); otherwise the applicant must, within 30 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 19 months.

See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the PCT Applicant's Guide, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the ISA/US
Commissioner for Patents
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3330

Authorized officer
Dorothea Lawrence
Sandra Sancier

Telephone No. (703) 308-0196

Form PCT/ISA/220 (April 2002)

(See notes on accompanying sheet)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference XY-IVF-PCT	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US01/43237	International filing date (day/month/year) 29 November 2001 (29.11.2001)	(Earliest) Priority Date (day/month/year) 29 November 2000 (29.11.2000)
Applicant XY, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 7 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the Report

- a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.



the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing:



contained in the international application in written form.



filed together with the international application in computer readable form.



furnished subsequently to this Authority in written form.



furnished subsequently to this Authority in computer readable form.



the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.



the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

2. ☐ Certain claims were found unsearchable (See Box I).

3. ☒ Unity of invention is lacking (See Box II).

4. With regard to the title,



the text is approved as submitted by the applicant.



the text has been established by this Authority to read as follows:

5. With regard to the abstract,



the text is approved as submitted by the applicant.



the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No. _____



as suggested by the applicant.



because the applicant failed to suggest a figure.



because this figure better characterizes the invention.



None of the figures

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/45237

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-19

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/43237

A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : A01N 1/00 US CL : 435/2 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/2 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) USPATFULL, MEDLINE, BIOSIS, WPMDS		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,119,465 A (MULLENS et al.) 19 september 2000.	I-19
A.P	US 6,238,920 B1 (NAGAI et al.) 29 May 2001.	I-19
☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another claim or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and which is an improvement on the application but does not understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "A" document member of the same patent family		
Date of the actual completion of the international search 19 April 2002 (19.04.2002)		Date of mailing of the international search report 14 MAY 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230		Authorized officer Sandra Sanchez Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/45237

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-19, drawn to a first method, a method of transporting oocytes.

Group II, claims 20-42, drawn to a second method, a method of fertilizing oocytes.

Group III, claim 43, drawn to a mammal of a preselected gender.

Group IV, claims 44-55, drawn to an incubation system.

Group V, claims 56-63 drawn to a third method, a method of in vitro fertilizing of oocytes. The inventions listed as Groups I-V do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I, II and V are directed to three different methods because they have distinct steps. Multiple methods are not permitted under PCT Rule 13.1.

Group III does not relate to a single general inventive concept under PCT Rule 13.2 because sex selection of mammals is well known in the prior art and further, the animal, per se, cannot be distinguished from an animal whose gender was not predetermined.

Group IV is not related to a single general inventive concept under PCT Rule 13.2 because the incubation system of Group IV where the temperature and CO₂ levels are stipulated is not required to be used in the methods of Groups I, II or V where the methods of claims 1, 20 or 56 do not stipulate any temperature or gas concentration.

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ US

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty.
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only		
Identification of IPEA		Date of receipt of DEMAND
Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference XY-IVF-PCT
International application No. PCT/US01/45237	International filing date (day/month/year) 29 November 2001	(Earliest) Priority date (day/month/year) 29 November 2000
Title of invention System for In-vitro Fertilization With Spermatozoa Separated Into X-Chromosome And Y-Chromosome Bearing Populations		
Box No. II APPLICANT(S)		
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) XY, Inc. 1108 North Lemay Avenue Fort Collins, Colorado 80524 United States of America		Telephone No. (970) 493-3113
		Facsimile No. (970) 493-3114
		Teleprinter No.
		Applicant's registration No. with the Office
State (that is, country) of nationality: US		State (that is, country) of residence: US
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) COLORADO STATE UNIVERSITY through its agent Colorado State University Research Foundation 601 South Howes, Room 410 Fort Collins, Colorado 80525 United States of America		
State (that is, country) of nationality: US		State (that is, country) of residence: US
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) SEIDEL, George E. 3101 Arrowhead Road Fort Collins, Colorado 80535 United States of America		
State (that is, country) of nationality: US		State (that is, country) of residence: US
☒ Further applicants are indicated on a continuation sheet.		

Continuation of Box No. II **APPLICANT(S)**

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

SUH, Tae Kwang
2044 Glenmoor Drive
Fort Collins, Colorado 80521
United States of America

State *(that is, country)* of nationality:
KR

State *(that is, country)* of residence:
US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

LU, Kehaun
14 Burrough Field
Impington, Cambridge CB4 9NW
United Kingdom

State *(that is, country)* of nationality:
UK

State *(that is, country)* of residence:
UK

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

☐ Further applicants are indicated on another continuation sheet.

Sheet No. 3

International application No.
PCT/US01/45237**Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE**The following person is ☒ agent ☐ common representativeand ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.Name and address: (Family name followed by given name; for a legal entity, full official designation.
The address must include postal code and name of country.)MILES, Craig R.
Santangelo Law Offices, P.C.
125 South Howes, Third Floor
Fort Collins, CO 80521
United States of America

Telephone No.

970-224-3100

Facsimile No.

970-224-3175

Teleprinter No.

Agent's registration No. with the Office

45,954

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.**Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION****Statement concerning amendments:***

1. The applicant wishes the international preliminary examination to start on the basis of:

☒ the international application as originally filedthe description ☒ as originally filed☐ as amended under Article 34the claims ☒ as originally filed☐ as amended under Article 19 (together with any accompanying statement)☐ as amended under Article 34the drawings ☒ as originally filed☐ as amended under Article 342. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.3. ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). (This check-box may be marked only where the time limit under Article 19 has not yet expired.)

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

☒ which is the language in which the international application was filed.☐ which is the language of a translation furnished for the purposes of international search.☐ which is the language of publication of the international application.☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.**Box No. V ELECTION OF STATES**

The applicant hereby elects all eligible States (that is, all States which have been designated and which are bound by Chapter II of the PCT)

excluding the following States which the applicant wishes not to elect:

None

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

- | | | |
|---|---|--------|
| 1. translation of international application | : | sheets |
| 2. amendments under Article 34 | : | sheets |
| 3. copy (or, where required, translation) of amendments under Article 19 | : | sheets |
| 4. copy (or, where required, translation) of statement under Article 19 | : | sheets |
| 5. letter | : | sheets |
| 6. other (specify) firm letter of transmittal and Express Mail Certificates for each document | : | sheets |

For International Preliminary Examining Authority use only

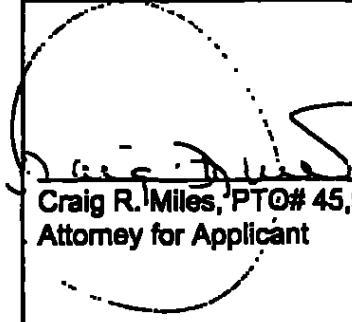
received	not received
☐	☐
☐	☐
☐	☐
☐	☐
☐	☐
☐	☐

The demand is also accompanied by the item(s) marked below:

- | | |
|--|--|
| 1. ☒ fee calculation sheet | 5. ☐ statement explaining lack of signature |
| 2. ☐ original separate power of attorney | 6. ☐ sequence listing in computer readable form |
| 3. ☐ original general power of attorney | 7. ☐ other (specify): |
| 4. ☐ copy of general power of attorney; reference number, if any: | |

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).


Craig R. Miles, PTO# 45,954
Attorney for Applicant

06-27-02
Date

For International Preliminary Examining Authority use only

- | | |
|--|---|
| 1. Date of actual receipt of DEMAND: | |
| 2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b): | |
| 3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. | ☐ The applicant has been informed accordingly. |
| 4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5. | |
| 5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 32. | |

For International Bureau use only

Demand received from IPEA on:

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

WRITTEN OPINION

(PCT Rule 66)

To: CRAIG R. MILES SANTANGELO LAW OFFICES, P.C. 185 SOUTH HOWES, THIRD FLOOR FORT COLLINS, COLORADO 80521		Date of Mailing (day/month/year) 14 NOV 2002
Applicant's or agent's file reference XY-IVF-PCT		REPLY DUE within TWO months from the above date of mailing
International application No. PCT/US01/45937	International filing date (day/month/year) 29 NOVEMBER 2001	Priority date (day/month/year) 29 NOVEMBER 2000
International Patent Classification (IPC) or both national classification and IPC IPC(7) A01N 1/00 and US CL: 435/8		
Applicant XY, INC.		

1. This written opinion is the first (first, etc.) drawn by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step or industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. ~~The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).~~

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 66.2 is: 29 MARCH 2003

Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20531	Authorized officer <i>Christopher Tate</i> CHRISTOPHER TATE
Facsimile No. (703) 305-3230	Telephone No. (703) 305-0196

WRITTEN OPINION

International application No.

PCT/US01/45237

L Basis of the opinion

1. With regard to the elements of the international application:*

☒ the international application as originally filed☒ the description:

pages 1-29 , as originally filed
 pages NONE , filed with the demand
 pages NONE , filed with the letter of

☒ the claims:

pages 30-63 , as originally filed
 pages NONE , as amended (together with any statement) under Article 19
 pages NONE , filed with the demand
 pages NONE , filed with the letter of

☒ the drawings:

pages 1-4 , as originally filed
 pages NONE , filed with the demand
 pages NONE , filed with the letter of

☒ the sequence listing part of the description:

pages NONE , as originally filed
 pages NONE , filed with the demand
 pages NONE , filed with the letter of

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language _____ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
☐ the language of publication of the international application (under Rule 48.3(b)).
☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the written opinion was drawn on the basis of the sequence listing:

- ☐ contained in the international application in printed form.
☐ filed together with the international application in computer readable form.
☐ furnished subsequently to this Authority in written form.
☐ furnished subsequently to this Authority in computer readable form.
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

- ☒ the description, pages NONE
☒ the claims, Nos. NONE
☒ the drawings, sheets/fig. NONE

5. ☐ This opinion has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".

WRITTEN OPINION

International application No.
PCT/US01/45837

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application.

☒ claims Nos. 20-63

because:

☐ the said international application, or the said claim Nos. _ relate to the following subject matter which does not require international preliminary examination (*specify*).

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. _ are so unclear that no meaningful opinion could be formed (*specify*).

☐ the claims, or said claims Nos. _ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 20-63.

2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

WRITTEN OPINION

International application No.
PCT/US01/48997

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims 1-19	YES
	Claims NONE	NO
Inventive Step (IS)	Claims 1-19	YES
	Claims NONE	NO
Industrial Applicability (IA)	Claims 1-19	YES
	Claims NONE	NO

2. citations and explanations

Claims 1-19 meet the criteria set out in PCT Article 33(8)-(4), because the prior art does not teach or fairly suggest a method of transporting oocytes comprising the claimed steps. The method is useful in transporting as well as selectively fertilizing oocytes prior to *in vitro* fertilization.

NEW CITATIONS
NONE

WRITTEN OPINION

International application No. PCT/US01/45937

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 1-19 are objected to under PCT Rule 26.5(a)(v) as lacking clarity under PCT Article 6 because the claims 1-19 are indefinite for the following reason(s): Many of the claims, including claim 1, recite the phrase "incubation element". It is unclear by the term "element" in this phrase as to what is actually being defined. For example, is this defining a singular element/component of an incubator, an element from the periodic table, or a device (having apertures, etc.). For clarity, it would seem more appropriate to instead recite --incubation device-- or --incubator--.

WRITTEN OPINION

International application No. PCT/US01/48227

Supplemental Box (To be used when the space in any of the preceding boxes is not sufficient)	
Continuation of: Boxes I - VIII	Sheet 10
TIME LIMIT: The time limit set for response to a Written Opinion may not be extended. 37 CFR 1.484(d). Any response received after the expiration of the time limit set in the Written Opinion will not be considered in preparing the International Preliminary Examination Report.	

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
CRAIG R. MILES
SANTANGELO LAW OFFICES, P.C.
125 SOUTH HOWES, THIRD FLOOR
FORT COLLINS, CO 80521

PCT
NOTIFICATION OF TRANSMITTAL OF
INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing
(day/month/year) **30 APR 2003**

Applicant's or agent's file reference

XY-IVF-PCT

IMPORTANT NOTIFICATION

International application No.

International filing date (day/month/year)

Priority date (day/month/year)

PCT/US01/43237

29 November 2001 (29.11.2001)

29 November 2000 (29.11.2000)

Applicant

XY, INC.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.
4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US

Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703)305-3230

Form PCT/IPEA/416 (July 1992)

Authorized officer

Christopher R. Tate
Christopher R. Tate

Telephone No. 703-308-0196

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference XY-IVR-PCT		FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US01/45237	International filing date (day/month/year) 29 November 2001 (29.11.2001)	Priority date (day/month/year) 29 November 2000 (29.11.2000)	
International Patent Classification (IPC) or national classification and IPC IPC(7): A01N 1/00 and US Cl.: 435/2			
Applicant XY, INC.			
1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36. 2. This REPORT consists of a total of 5 sheets, including this cover sheet. ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT). These annexes consist of a total of 0 sheets. 3. This report contains indications relating to the following items: I ☒ Basis of the report II ☐ Priority III ☒ Non-establishment of report with regard to novelty, inventive step and industrial applicability IV ☐ Lack of unity of invention V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI ☐ Certain documents cited VII ☐ Certain defects in the international application VIII ☒ Certain observations on the international application			
Date of submission of the demand 27 June 2002 (27.06.2002)		Date of completion of this report 06 March 2003 (06.03.2003)	
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230		Authorized officer  Christopher R. Tate Telephone No. 703-308-0196	

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/USD1/45237

I. Basis of the report

1. With regard to the elements of the international application:*

- ☒ the international application as originally filed.
- ☒ the description:
 pages 1-29 as originally filed
 pages NONE, filed with the demand
 pages NONE, filed with the letter of _____.
- ☒ the claims:
 pages 30-63 as originally filed
 pages NONE, as amended (together with any statement) under Article 19
 pages NONE, filed with the demand
 pages NONE, filed with the letter of _____.
- ☒ the drawings:
 pages 1-4 as originally filed
 pages NONE, filed with the demand
 pages NONE, filed with the letter of _____.
- ☐ the sequence listing part of the description:
 pages NONE, as originally filed
 pages NONE, filed with the demand
 pages NONE, filed with the letter of _____.

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language _____ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in printed form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages NONE
- ☐ the claims, Nos. NONE
- ☐ the drawings, sheets/fig NONE

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(e)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US01/45237

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The question whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application,

☒ claims Nos. 20-63

because:

☐ the said international application, or the said claim Nos. _____ relate to the following subject matter which does not require international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. _____ are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 20-63

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US01/45237

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims <u>1-19</u>	YES
	Claims <u>NONE</u>	NO
Inventive Step (IS)	Claims <u>1-19</u>	YES
	Claims <u>NONE</u>	NO
Industrial Applicability (IA)	Claims <u>1-19</u>	YES
	Claims <u>NONE</u>	NO

2. CITATIONS AND EXPLANATIONS

Claims 1-19 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a method of transporting oocytes comprising the claimed steps.

Claims 1-19 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

NEW CITATIONS

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US01/45237

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the questions whether the claims are fully supported by the description, are made:

Claims 1-19 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because claims 1-19 are indefinite for the following reason(s): Many of the claims, including claim 1, recite the phrase "incubation element". It is unclear by the term "element" in this phrase as to what is actually being defined. For example, is this defining a singular element/component of an incubator, an element from the periodic table, or a device (having apertures, etc.). For clarity, it would seem more appropriate to instead recite --incubator device-- or --incubator--.

**DISPOSICION PARA FERTILIZACION IN-VITRO CON ESPERMATOZOIDES
SEPARADOS DENTRO DE POBLACIONES QUE CONTIENEN CROMOSOMA X Y
CROMOSOMA Y**

Esta solicitud reivindica el beneficio de la solicitud de patente provisional de Estados Unidos N° 60/253,787 presentada en noviembre 29, 2000 y la solicitud de patente provisional de los Estados Unidos N° 60/253,785 presentada en noviembre 29, 2000, cada una de las cuales es incorporada a la presente a modo de referencia.

I. CAMPO TECNICO

Dispositivos, composiciones y métodos que mejoran la calidad de los embriones generados utilizando fertilización in-vitro (IVF) con espermatozoides separados en poblaciones que contienen cromosoma X y cromosoma Y.

II. ANTECEDENTE

Una característica atractiva del IVF es que muy pocos espermatozoides pueden ser requeridos para la inseminación a diferencia de la inseminación artificial. Sin embargo, el IVF utiliza espermatozoides separados dentro de poblaciones que contienen cromosoma X y cromosoma Y (espermatozoide separado) puede necesitar modificaciones con respecto a las técnicas convencionales IVF. Esto puede deberse en parte a la pre-capacitación de tal espermatozoide.

En la mayoría de los casos, el porcentaje de oocitos (oocito, ootide, u óvulo, o una pluralidad de los mismos tal como es apropiado para la solicitud) fertilizados con espermatozoides separados y sin separar son similares, y los eventos durante el primer ciclo de célula son cronometrados similarmente para los espermatozoides separados y sin separar.

Sin embargo con procedimientos convencionales, la producción del blastocisto con espermatozoide separado puede ser del 70%-90% de controles con espermatozoide que no han sido separado. Por ejemplo, el desarrollo de blastocisto ha mostrado ser del 17% con oositos bovinos inseminados con espermatozoides separados, comparado con >25% el cual puede esperarse con el IVF utilizando espermatozoide sin separar tal como se describe en el artículo jornal intitulado "Fertilización in-vitro con esperma bovino seleccionado por citometría de flujo" Teriogeneologia 52: 1393, 1405 (1999), incorporado a la presente a modo de referencia.

Varios factores pueden contribuir a estos resultados. Un factor puede ser aquel que pigmenta el esperma con Hoechst 33342 aparentemente causa una disminución en la movilidad del espermatozoide. Otro factor puede ser la fuerzas físicas a la que los espermatozoides son sometidos durante el proceso de separación. Como un ejemplo, en la separación por citometría de flujo del espermatozoide, el mismo sale del citómetro de flujo a casi 100 km/h previo a impactar sobre la superficie del medio recolector. Durante el transito a través del citómetro de flujo el espermatozoide puede ser sometido a una luz láser de una intensidad sobre 100mW. Mientras que el tiempo de transito puede ser solo de 1-2µsec, esto puede afectar el ADN del espermatozoide, y de esta manera también afectar subsecuentemente el desarrollo embrionario. El proceso de separar esperma con citometría de flujo puede dar como resultado una muestra altamente diluida, 600.000 espermatozoides/mL o menos, y los subsiguientes pasos de centrifugado son necesarios para proveer espermatozoides concentrados adecuados para la inseminación.

Otro problema con la utilización de espermatozoides en técnicas de IVF puede ser que la facilidad en la cual el

espermatozoide es separado puede ser en una ubicación diferente que en donde el mamífero hembra de la cual son recolectados estén ubicados, los cuales pueden ser diferentes desde donde el mamífero hembra desde la cual los oositos son recolectados, estén ubicados, los cuales pueden estar en una ubicación diferente desde donde la fertilización in-vitro da a lugar, y la cual puede ser una ubicación diferente desde donde el mamífero hembra dentro del cual los embriones cultivados in-vitro serán transferidos. Convencionalmente, el esperma separado puede ser crio-preservado y transportado congelado hacia la ubicación en donde la técnicas IVF son administradas. Los oositos en maduración son convencionalmente transportados hacia el establecimiento en el cual las técnicas de IVF son administradas, siendo estos sistemas de incubación portátiles. Los oositos en maduración son luego inseminados con células de esperma previamente congelado-descongelado. Para evitar la crio preservación de las células de esperma o como una conveniencia para las varias instalaciones incorporadas, puede ser beneficioso transportar los oositos en maduración directamente hacia el establecimiento de separación del espermatozoide, para que las células de esperma separadas puedan ser agregadas a los oositos sin la crio preservación. Sin embargo, el IVF convencional y la cultura in-vitro de la cigota resultante generalmente comprende un juego separado de aparatos y procedimientos que hacen inconveniente, difícil o imposible inseminar y cultivar oositos de la misma manera en que se separan los espermatozoides.

Incluso cuando los espermatozoides que contiene cromosoma X y cromosoma Y han sido diferenciados y separados basados sobre la diferencia de la fluorescencia emitida, durante varios años, e incluso cuando el espermatozoide

separado ha sido utilizado durante algún tiempo con las técnicas IVF, e incluso cuando existe un gran mercado comercial para embriones producidos con técnicas IVF y espermatozoides separados, los problemas mencionados anteriormente todavía no han sido resueltos.

Con respecto a los problemas con técnicas convencionales utilizando espermatozoides separados, y específicamente espermatozoide separado, espermatozoide pigmentado, o espermatozoide obtenido de esperma previamente congelado, y con las estrategias convencionales que incorporan el transporte de esperma separado y oositos en maduración, la invención se dirige a cada uno en una manera practica.

III. DIVULGACION DE LA INVENCION

De esta manera, uno de los amplios objetos de las realizaciones particulares de la invención, puede ser proveer dispositivos, composiciones y métodos que proveen el transporte de oositos inseminados, promueve la fisura de oositos fertilizados y mejora la calidad de los embriones generados con técnicas que utilizan espermatozoides separados en poblaciones que contienen cromosoma X y cromosoma Y

Otro amplio aspecto de las realizaciones particulares de la invención pede ser proveer dispositivos, composiciones y métodos que promueven la fisura y mejoran la calidad de embriones generados utilizando IVF con espermatozoides que son derivados de esperma previamente congelados.

Otro objeto amplio de las realizaciones particulares de la invención puede ser proveer dispositivos composiciones y métodos que promueven la fisura y mejoran la calidad de los embriones generados utilizando IVF con espermatozoides que

han sido previamente pigmentados con ADN unido con fluorocromo

Otro amplio aspecto de las relaciones particulares de la invención pueden ser proveer medios para el cultivo embrionario que puede contener aminoácidos no esenciales.

Otro amplio aspecto de la invención puede ser el proveer aparatos y métodos para transportar oocitos en maduración y oocitos fertilizados para la conveniencia del usuario final o para evitar la criopreservación del espermatozoide utilizado en ovositos fertilizados.

Los objetos naturalmente posteriores de la invención son divulgados a través de otras áreas de la memoria.

IV BREVE DESCRIPCIÓN DE LOS DIBUJOS

La figura 1 muestra una recesión de la invención en donde los espermatozoides son pigmentados a partir de esperma fresco o previamente congelado y descongelado.

La figura 2 muestra una realización de la invención para separar espermatozoides pigmentados en poblaciones que contienen cromosomas x y cromosomas y.

La figura 3 muestra otra vista de una realización de la invención para separar espermatozoides pigmentados en poblaciones que contiene cromosomas x y cromosomas y.

La figura 4 muestra una realización de la disposición de incubación portátil en donde los ovositos pueden ser fertilizados.

V MODOS PARA LLEVAR A CABO LA INVENCION

La invención incluye dispositivos, métodos y composiciones para la inseminación en vitro y la fertilización de ovositos (ovosito, ootides, u óvulos o la pluralidad de los mismos tal como sea apropiado para la

solicitud) y el cultivo de embriones resultando de tales técnicas.

Las realizaciones de la invención pueden incluir espermatozoides frescos, o espermatozoides a partir de esperma congelado y descongelado de varias especies de mamíferos. La invención puede ser interpretada no estando limitada por las especies de mamíferos citados por los ejemplos específicos dentro de esta solicitud de patente. Las realizaciones de la invención, por ejemplo pueden incluir espermatozoide fresco o espermatozoide a partir de esperma congelado y descongelado de animales que presentan para los comerciales de carne o productos diarios o lácteos tales como porcinos, bovinos, equinos, búfalo o lo similar (naturalmente los mamíferos utilizados para carne o productos diarios o lácteos que pueden variar de cultura a cultura). Puede además incluir espermatozoide fresco o espermatozoide a partir de esperma congelado - descongelado a partir de individuales que presentan atributos raros o no comunes, tales como características morfológicas que incluyen peso tamaño o conformación u otras características deseadas tales como velocidad, agilidad, intelecto o lo similar. Pueden incluir esperma congelado - descongelado a partir de donantes muertos, o espermatozoide fresco o congelado - descongelado a partir de mamíferos raros o exóticos, tal como especímenes zoológicos o especies en peligro de extinción. Las realizaciones de la invención pueden además incluir espermatozoide fresco o congelado y descongelado recolectado de primates, incluyendo pero no estando limitado a, humanos chimpancés, gorilas y lo similar y puede además incluir espermatozoide fresco o congelado y descongelado a partir de mamíferos marinos tales como ballenas, marsopas y delfines.

Haciendo ahora referencia principalmente a la figura 1, en algunas realizaciones de la invención, la pigmentación a partir de Hoechst 33342 (1) puede ser agregado al espermatozoide bovino contenido en el esperma congelado - descongelado (2) para establecer una concentración de 224 μM . El tiempo de incubación del espermatozoide contenido en el esperma congelado - descongelado (2) con la pigmentación (1) puede ser alrededor de 190 minutos. En otra realización de la invención, la pigmentación (1) puede ser agregada al esperma bovino (2) para establecer una concentración de 2240 μM y luego incubado por un tiempo de 60 minutos. El esperma congelado - descongelado tratado de cualquier manera puede mejorar la resolución del espermatozoide que contiene en cromosoma x y el cromosoma y. Se puede entender, que de aplicación a aplicación (tal como esperma congelado - descongelado de diferentes especies) la cantidad de tiempo de incubación y la concentración específica del pigmento puede ajustarse para optimizar la resolución de espermatozoide que contiene cromosoma x y cromosoma y.

Con respecto a los promedios de fisura de los oocitos inseminados, el incremento en la concentración de pigmentación hasta al menos 10 x no aparenta tener un efecto depresivo en ya sea la fisura o el desarrollo embrionario. A mayores concentraciones de pigmentación puede actualmente ser benéfico con respecto a ciertas aplicaciones debido a la longitud de ciertos del tiempo de incubación que puede ser reducido mejorando el porcentaje de fisura. De aplicación a aplicación la longitud del tiempo de incubación puede ser ajustado para optimizar los resultados de fisura o el desarrollo embrionario tal como se desee.

Haciendo ahora referencia principalmente a las figuras 2 y 3, una realización de citómetro de flujo de la

invención es ilustrado el cual incluye una fuente de célula de esperma 13 el cual actúa para establecer o suministrar espermatozoides pigmentados u otro tipo de células pigmentadas para ser analizadas por el citómetro de flujo. Las células de esperma son depositadas dentro de una boquilla (4) de una manera tal que las células son rodeadas por una vaina de fluido (5). La vaina de fluido (5) está generalmente suministrada por alguna fuente de vaina de fluido (6) para que mientras la fuente de célula (3) suministra la célula de esperma, el fluido de vaina (5) está concurrentemente alimentado a través de la boquilla (4). En esta manera puede ser fácilmente entendido como la vaina de fluido (5) forma un medio ambiente de vaina de fluido para las células. Dado que varios fluidos son provistos al citómetro de flujo con algo de presión, estos fluyen fuera de la boquilla (4) y salen del orificio de boquilla (7). Proveyendo algún tipo de oscilador (8) puede ser controlado muy precisamente a través de un control de oscilador (9), las ondas de presión pueden ser estabilizadas dentro de la boquilla (4) y transmitida a los fluidos que salen de la boquilla en el orificio de boquilla (7). Dado que el oscilador (9) de esta manera actúa sobre la vaina de fluido (5), la corriente (10) sale del orificio de boquilla (7) eventualmente y regularmente forma gotas (11). Debido a que las células de esperma son rodeadas por una vaina de medio ambiente de vaina de fluido, las gotas (11) pueden contenerse dentro de células aisladas individualmente (generalmente) u otros ítems.

Dado que las gotas (11) generalmente contienen de esperma aisladas, el citómetro de flujo puede distinguir y separar las gotas basadas sobre ya sea o no la célula de esperma apropiada que esté contenida dentro de la gota. Esto es realizado a través de una disposición de detección de

célula (12). La disposición de detección de célula incorpora al menos algún tipo de sensor (13) el cual responde a las células contenidas de cada botón tal como se describe en la Patente Norteamericana US No. 5135759, incorporada a la presente a modo de referencia. Mientras que la patente de Johnson se basa sobre el espermatozoide o célula de esperma, aunque la pigmentación y las invenciones de separación puede ser entendidas para ser utilizadas con una variedad de células congeladas - descongeladas, la disposición de detección de célula (13) puede causar una acción dependiendo sobre la presencia relativa o la ausencia relativa de fluorocromo incorporado el cual puede ser excitado por algún estimulante tal como un excitador láser (14). Mientras que cada tipo de célula de esperma puede pigmentado por un pigmento o fluorocromo, tal como fue descrito anteriormente, las diferentes longitudes del cromosoma x y el cromosoma y causan diferentes cantidades de pigmentos a ser unidos. De esta manera, detectando el grado de fluorescencia emitida por el fluorocromo luego de la excitación es posible discriminar entre los espermatozoides que contienen x y los espermatozoides que contienen y por sus diferentes niveles de emisión de fluorescencia.

Para realzar la separación y aislación de las células de esperma apropiadas, las señales recibidas por el sensor (14) son alimentadas a algún tipo de disposición de discriminación de selección (15) el cual rápidamente realiza una decisión de diferenciación y puede diferencialmente cargar cada gota (11) basada sobre ya sea si ha decidido que la célula de esperma deseada sale o no sale con aquella gota (11). De esta manera la separación o disposición de discriminación (15) actúa para permitir las placas de deflexión electroestáticas (16) para que desvíen las gotas

(11) basadas en ya sea o no que estas contengan la células de esperma apropiadas. Como resultado, el citómetro de flujo actúa para seleccionar las células de esperma causando que las mismas aterricen en una o más colectores (17) de esta manera detectando alguna propiedad de la célula de esperma del citómetro de flujo puede discriminar entre las células de esperma basadas en una característica particular y ubicar las mismas en un recolector apropiado (17). En la disposición presente utilizada para seleccionar espermatozoides, las gotas de espermatozoides que contienen cromosoma x son cargadas positivamente y de esta manera se desvían en una dirección, y las gotas de espermatozoide que contienen el cromosoma y son cargadas negativamente y de esta manera se desvían hacia el otro lado, y en donde la corriente de desperdicio (la que contiene células sin seleccionar) no es cargada y de esta manera es recolectada en una corriente sin desviar dentro de un tubo de succión o lo similar.

Haciendo ahora referencia principalmente a la figura 3, el proceso puede ser incluso entendido de mejor manera. Tal como se ilustra en aquella figura, la boquilla (4) emite una corriente (10) la cual debido al oscilador (8) no ilustrado en la figura 3) forma las gotas (11). Dado que la fuente de célula (3) (no ilustrada en la figura 3) puede suministrar células de esperma (1) las cuales han sido pigmentadas de acuerdo con la invención, la magnitud de la emisión fluorescente estimulada por el excitador láser (13) es determinado diferencialmente por un sensor (14) para que la existencia o la no existencia de una carga en cada gota (11) sea separado de la corriente (10) que puede ser controlada por el citómetro de flujo. Este control da como resultado una carga positiva, una carga negativa y gotas sin cargar basado sobre la célula de esperma encapsulada. Tal

como se dicta en la figura 3, ciertas gotas son ilustradas como gotas desviadas (18). Estas gotas desviadas (18) son aquellas que contienen células de esperma (2) diferenciadas por el contenido de cromosoma x o cromosoma y. Los espermatozoides separados son luego depositados en un recolector apropiado (17) para su uso posterior. Ver además la solicitud de Patente Internacional PCT/US98/27909, incorporada a la presente a modo de referencia.

Mientras que la descripción anterior se enfoca en la separación de espermatozoides con el citómetro de flujo, la separación de espermatozoides que contienen el cromosoma x y espermatozoides que contienen el cromosoma y basados sobre la diferencia en la medición de la ignición de fluorescencia puede además incluir varias otras tecnologías tales como la cromatografía líquida, electroforesis de gel, y otras tecnologías que generalmente excitan la cantidad de fluorocromo incorporado para diferenciar entre el espermatozoide que contiene el cromosoma x y el espermatozoide que contiene el cromosoma y.

Las retenciones de la invención también pueden comprender la recolección de oocitos a partir del mamífero hembra. Con respecto a ciertas realizaciones de la invención, los oocitos pueden ser aspirados desde los ovarios del mamífero hembra deseado o pueden ser obtenidos a partir del matadero de ovarios. Los oocitos pueden ser madurados en TCM 199 suplementados con alrededor de 10% de suero de ternero fetal de 10% más las hormonas (15 ng FSH, 1 μ g LH, 1 μ g E_2 /ml) durante 22 a 24 horas a 39 °C, en alrededor de 5% de CO_2 en aire.

Diez de los 15 oocitos pueden ser transferidos a una gota de 50 μ l de medio de fertilización que contiene aminoácidos no esenciales, tal como piruvato lactate albumin

tiroides (TALP) suplementado con aminoácidos no esenciales derivados de águilas medianas, y en donde además puede contener 0.6% de suero bovino de albúmino, 29 μ g de heparina/mL y 5 mM de cafeína. Alternativamente, los oocitos pueden ser fertilizados en otros medios que contienen aminoácidos no esenciales tales como el medio definido químicamente en el artículo jornal titulado "Tensión de oxígeno reducida y EDTA mejorado del desarrollo de zygote bovino en un medio definido químicamente", J Anim Sci (1999), o el medio SOF descrito en el artículo jornal "cultura in vitro exitosa de óvulo de ganado y ovejas", J Reprod. Fertil. 30:493-497 (1972), cada artículo del jornal incorporado a la presente a modo de referencia.

Luego de separar o seleccionar, la célula de espermatozoos puede ser lavada por centrifugado durante 10 minutos a 400 g en el medio de recolección (generalmente un medio de piruvato lactato de albúmina hepes-tiroide suplementado con 2.0% suero bovino de albúmina) seguido por la suspensión en el medio de fertilización. El espermatozoos seleccionado descongelado puede ser preparado por centrifugado durante 20 minutos a 700 g a través de un percoll graduado (90%: 45%) para la separación de espermatozoos vivos y muertos. El pellet de espermatozoos puede ser luego lavado con el medio de fertilización por el centrifugado a 400 g durante 10 minutos. El espermatozoos puede ser luego agregado al medio de fertilización para dar una concentración de 1 a 2 millones/mL.

Tabla 1. Etapa de fisura del ovocito inseminado con espermatozoos separado en cuatro medios de fertilización diferentes.

Medios	No.	5 segment.	2-célula	8 célula
--------	-----	------------	----------	----------

	oocitos		a 24 h	a 72 h
Fert-TALP	168	76	6 ^a	66
Fert-TALP + a.c.n.e.	176	71	26 ^b	67
CDM	167	89	75 ^c	70
SOF	145	86	49 ^d	69

^{a,b,c,d} Medios con diferentes superíndice que difieren (P<.05) .

Haciendo ahora referencia principalmente a la tabla 1, tal como puede entenderse, los oocitos inseminados con espermatozoide separado en un medio de fertilización que contiene aminoácidos no esenciales de acuerdo con la invención exhiben un promedio incrementado de un desarrollo temprano a través de al menos dos etapas de células.

Tabla 2. Desarrollo embrionario y calidad de blastocito resultante de la fertilización en cuatro medios de fertilización diferentes (promediado sobre dos medios de cultivo).

Medios	No. Oocitos	%blastocitos/oocitos		%grado 1 blastocitos/total de blastocitos
		Total	D7	
Fert-TALP	326	20	17	52 ^{a,c}
Fert-TALP -aa	221	20	17	68 ^b
CDM	332	22	18	61 ^{b,c}
SOF	321	21	17	64 ^{b,c}

^{a,b,c} Porcentajes sin superíndice diferentes comunes
($P < .05$)

^d Grado 1 indica los blastocistos con una masa de célula interior distinta adecuada para la transferencia de embriones.

Haciendo referencia ahora principalmente a la tabla 2, alguna realizaciones de la invención en la cuales los oocitos son fertilizados con espermatozoides seleccionados en un medio de fertilización contienen aminoácidos no esenciales suplementados que exhiben una calidad mejorada de embriones. En las realizaciones de la invención en la cual oocitos fueron fertilizados en un piruvato lactatio alumini tiroides (TALP) suplementado con aminoácidos no esenciales derivados de águilas medianas, contienen además 0.6% de albúmina de suero bovino, 20 μ g de heparina/mL y 5 mM de cafeína que fueron diferenciadas ($P < .05$) en la calidad de los embriones comparados con el TALP son los aminoácidos no esenciales.

Los supuestos cigotes pueden ser removidos del cultivo y ubicados en un medio definido químicamente (CDM-1) tal como se discute en el jornal de ciencia animal, 78, 152-157 (2000), incorporado a la presente a modo de referencia, durante 6 - 7 horas luego de la inseminación y cultivado durante 65 a 66 horas. Los embriones que son segmentados fueron además cultivado durante 96 horas en un CDM 2 (además contenía MEM esencial y aminoácidos no esenciales y 2.0 mM de fructuosa) que contiene 0.12 IU de insulina/mL. Los blastocistos fueron graduados morfológicamente de acuerdo con el tamaño de la masa de célula interna y pigmentados con Giemsa para determinar el número de células en el séptimo día luego de la inseminación.

Haciendo ahora referencia a la figura 4, la invención además incorpora una disposición de incubación portátil. Ciertas realizaciones de la invención pueden comprender una cánula (19) que presenta un volumen interior entre alrededor de 0.1 mL y alrededor de 0.5 mL dentro del cual el medio de fertilización, y los oocitos recolectados del miembro hembra, pueden ser transferidos. Mientras que la cánula 19 puede ser hecha de cualquier material compatible con el medio de fertilización y los oocitos recolectados, la realización específicas de la cánula (19) pueden ser hechas de plástico, tal como o similar a una cánula de inseminación artificial. Los extremos de la cánula de plástico pueden ser sellados por calor luego del medio de fertilización y los oocitos son transferidos ahí dentro.

La invención además puede comprender un elemento de incubación (20) configurado para encapsular la cánula (19) o una pluralidad de cánulas insertadas dentro de la misma. En algunas realizaciones de la invención el elemento de incubación (20) puede ser un tubo de vidrio que presenta un elemento de aberturas sellable únicamente, el elemento de abertura (21) puede ser sellado con una tapa (22), y en algunas realizaciones la tapa (22) y el tubo pueden tener roscas espirales (23) que pueden ser roscados por giro para cerrar el elemento de incubación (20).

Luego de transferir una cánula (19) o una pluralidad de cánulas al volumen interior del elemento de incubación (20) las condiciones de incubación pueden ser establecidas dentro de las mismas. Las condiciones de incubación típicas dentro del volumen interior del elemento de incubación puede comprender una atmósfera de 5% de dióxido de carbono en aire y una temperatura de alrededor de 39 °C (37 °C a 41°C). Una vez que las condiciones de incubación son establecidas dentro

del elemento de incubación, el elemento de incubación (20) puede ser sellado y los oocitos pueden ser luego transportados dentro del elementos de incubación (20).

En algunas realizaciones de la invención, los oocitos pueden ser transportados a una instalación de separación de célula de esperma en donde el elemento de incubación (20) es abierto, la cánula (19) es abierta y una pluralidad de célula de esperma (15) a partir de una población separada en base de el contenido de cromosoma x y contenido de cromosoma y puede ser transferido dentro de la cánula (19) conteniendo los oocitos. Con respecto a algunas realizaciones de la invención una concentración de células de esperma separadas (15) pueden ser establecidas dentro de alrededor de un millón a alrededor de dos millones/mL de medios de fertilización. La cánula (19) que contiene los oocitos y el espermatozoide en el medio de fertilización pueden ser luego resellados y transferidos nuevamente al elemento de incubación (20). Las condiciones de incubación pueden ser reestablecidas y el elemento de incubación sellado nuevamente. El elemento de incubación (20) que contiene una cánula o una pluralidad de cánulas (19) pueden luego ser transportado. Durante el transporte los oocitos pueden ser fertilizados. Luego del arribo los cigotes pueden ser transferidos desde la cánula para un cultivo posterior. Con respecto a ciertas realizaciones de la invención, lo oocitos pueden ser primeramente inseminados con espermatozoides separados o sin separar en cuotas convencionales de 50 mL y cargados en una cánula o cánulas de 0.25 mL (19) dentro de dos horas luego de la inseminación. Las cánulas (19) pueden ser selladas por calor y puestas en el elemento de incubación (20). El elemento de incubación abierto contiene cánulas con oocitos inseminados que pueden ser equilibrados con 5% de dióxido de carbono en aire y

alrededor de 39 °C durante al menos una hora y luego tapados a presión y cultivados bajo las mismas condiciones durante alrededor de 18 a 20 horas.

Haciendo referencia nuevamente a la tabla 2, muy pocos oocitos ($P < 0.05$) fertilizados en el Fert-TALP desarrollaron a la etapa de dos células durante 24 horas que en cualquier otro medio. Notablemente, la vasta mayoría de oocitos (75%) fertilizados en un medio CDM segmentados a la etapa de dos células por este tiempo. Luego de 72 horas de la post inseminación, no existió diferencia entre cualquiera de los medios, posiblemente debido a la tapa larga de 8 células del ciclo de células.

No existe diferencia entre cualquiera de los medios en el promedio de desarrollo al blastocistos. Sin embargo, existió una diferencia significativa en la calidad de los embriones entre el Fert-TALP y el Fert-TALP + los aminoácidos no esenciales.

La progresión del desarrollo embrionario temprano del bovino utilizando espermatozoides separados son similares a los estudios con la fisura in vivo o in vitro de oocitos fertilizados con espermatozoides sin separar. En la vaca, la primer fisura in vivo sucede a las 24-28 horas siguientes de la ovulación, y la primer fisura in vitro toma lugar a las 24-48 horas luego de la inseminación.

La fisura temprana sucedió con los oocitos fertilizados en un medio CDM, SOF y Fert-TALP + aa que en el medio convencional Fert-TALP. Esto puede ser debido a que el CDM, SOF, y el Fert-TALP + los aminoácidos no esenciales contenían todos aminoácidos no esenciales los cuales pueden jugar un rol en como el espermatozoide penetra rápidamente el oocito, en la longitud del primer ciclo de célula.

Tal como puede ser fácilmente entendido a partir de lo mencionado anteriormente, el concepto básico de la presente invención puede ser realizado en la variedad de modo. Incorpora la pigmentación de espermatozoide en donde el espermatozoide fresco o espermatozoide congelado - descongelado, la separación y técnicas de aleación las cuales pueden ser utilizadas con tales espermatozoides pigmentados, así como también dispositivos para realizar la pigmentación, separación, aislación de tales espermatozoides pigmentados dentro de poblaciones que contienen cromosoma x y cromosoma y, y el transporte de oocitos en maduración y oocitos fertilizados. En esta solicitud de patente, el pigmentado y las técnicas de separación utilizados con espermatozoides son divulgados como parte del resultado ilustrado para ser alcanzado por varios dispositivos descritos y mientras que los pasos son inherentes a la utilización. Estos son simplemente los resultados naturales de la utilización del dispositivo tal como son intencionados y descritos. Además, mientras que algunos dispositivos son divulgados debe entenderse que estos no sólo realizan ciertos métodos que además pueden ser variados en un número de modos. Es importante con respecto a todo lo antedicho, que todas estas facetas deben ser entendidas para ser abarcada por esta divulgación.

La discusión incluida en esta solicitud de patente del Tratado de Cooperación de Patente internacional es su intención servir como descripción básica. El lector debe ser consciente de que la discusión específica no puede ser explícitamente descrita con todas las realizaciones posibles; varias alternativas son implícitas. También puede no aplicar completamente la naturaleza genérica de la invención y puede no mostrar explícitamente como cada característica o elemento

puede actualmente ser representativo de una función más amplia o de una mayor variedad de alternativas o elementos equivalentes. Nuevamente, estos son implícitamente incluidos en esta divulgación. En donde la invención es descrita en base a la terminología orientada funcionalmente, cada aspecto de la función es realizado por un dispositivo su retina o programa. Las reivindicaciones del aparato pueden no sólo ser incluidas para el dispositivo descrito, pero además las reivindicaciones de métodos o procesos pueden ser incluidos para dirigir las funciones de la invención y cada elemento que lo realiza. Ninguna de las descripciones ni la terminología está dirigida a limitar el alcance de las reivindicaciones las cuales ahora son incluidas.

Además, cada uno de los varios elementos de la invención y reivindicaciones pueden además ser alcanzados de una variedad de maneras. Esta divulgación debe ser entendida para abarcar tal cada variación, puede ser una variación de una realización o cualquier realización de aparato, un método o realización de proceso, o incluso meramente una variación de cualquiera de los elementos de estos. Particularmente, debe entenderse que mientras la divulgación se refiere a elementos de la invención, las palabras para cada elemento puede ser expresado por términos de aparatos equivalentes o términos de métodos, incluso si sobre la función el resultado es el mismo. Tales términos equivalentes más amplios o incluso más genéricos deben ser considerados para que abarquen en la descripción de cada elemento u opción. Tales términos pueden ser sustituidos cuando se lo desea para hacer explícito la cobertura de amplitud implícita a la cual esta invención está dirigida. Como un ejemplo debe entenderse que todas las acciones pueden ser expresadas como medio para tomar aquella acción o como un elemento el cual realiza esa

acción. Similarmente, cada elemento físico divulgado debe ser entendido para que abarque una divulgación de la acción la cual ese elemento físico facilita. Respecto de este último aspecto y sólo como un ejemplo, la divulgación de un seleccionador debe ser entendido para que abarque la divulgación del acto de seleccionar, en donde explícitamente esté discutido o no, y a su vez en donde sólo la divulgación del acto de seleccionar tal como una divulgación debe ser entendida para abarcar la divulgación de un seleccionador o incluso un medio para seleccionar. Tales cambios y términos alternativos deben ser entendidos para que sean incluidos explícitamente en la descripción.

Adicionalmente, varias combinaciones y permutaciones de todos los elementos o aplicaciones pueden ser creadas y presentadas. Todas pueden ser realizadas para optimizar el diseño o rendimiento en una solicitud específica.

Cualquier acto de acto de ley estatuto regulación o reglas mencionadas en esta solicitud para la patente: o patentes publicaciones u otras referencias mencionadas en esta solicitud para patente son incorporadas a la presente a modo de referencia. Específicamente, la Solicitud de Patente Provisional US No. 60/253787, presentada en Noviembre 29, y 2000 y la Solicitud de Patente Provisional de Estados Unidos US No. 60/253785 presentada el 29 de Noviembre de 2000 son incorporadas a la presente a modo de referencia incluyendo cualquier figura o adjunto, cada una de las referencias en la siguiente tabla de referencias son incorporadas a la presente a modo de referencia.

La bibliografía que se menciona en la presente será respetada en su idioma original para facilitar la identificación de la misma.

Documentos de Patente de EE.UU.

DOCUMENTO N°	FECHA	NOMBRE	CLASE	SUBCLAS E	FECHA PRESEN.
32,350	02/10/ 87	Bhattachary a			11/22/74
3,687,806	08/29/ 72	Van den Bovenkamp	195	1.3	11/04/69
3,829,216	08/13/ 74	Persidsky	356	36	10/02/72
3,894,529	07/15/ 75	Shrimpton	128	1 R	04/10/69
4,009,260	02/22/ 77	Ericsson	424	105	12/11/74
4,067,965	01/10/ 78	Bhattachary a	424	105	12/17/75
4,083,957	04/11/ 78	Lang	424	78	02/04/76
4,085,205	04/18/ 78	Hancock	424	105	01/24/77
4,092,229	05/30/ 78	Bhattachary a	204	180 R	10/20/76
4,155,831	05/22/ 79	Bhattachary a	207	299 R	02/23/78
4,191,749	03/04/ 80	Bryant	424	105	10/11/77
4,225,405	09/30/ 80	Lawson	204	180 R	08/16/78
4,276,139	06/30/ 81	Lawson	204	180 R	10/09/79
4,339,434	07/13/ 82	Ericsson	424	105	08/17/81
4,362,246	12/07/ 82	Adair	209	3.3	07/14/80
4,448,767	05/15/ 84	Bryant	424	85	02/15/80
4,474,875	10/02/ 84	Shrimpton	435	002	08/18/80
4,501,366	02/26/ 85	Thompson	209	556	12/14/82
4,511,661	04/16/ 85	Goldberg	436	503	12/30/83
4,605,558	08/12/ 86	Shrimpton	424	561	04/20/84
4,660,971	04/28/ 87	Sage et al.	356	39	05/03/84
4,680,258	07/14/ 87	Hammerling et al	435	7	08/09/83
4,673,288	06/16/ 87	Thomas et al.			
4,683,195	07/28/ 97	Mullis et al			
4,683,202	07/28/ 87	Mullis			
4,698,142	10/06/ 87	Muroi et al	204	182.3	07/31/85
4,749,458	06/07/ 88	Muroi et al	204	182.3	03/02/87
4,790,653	12/13/ 88	North, Jr.			
4,988,619	01/29/ 91	Pinkel	435	30	11/30/87

4,999,283	03/12/91	Zavos et al	435	2	08/18/89
5,021,244	06/04/91	Spaulding	424	561	05/12/89
5,055,393	10/08/91	Kwoh et al			
5,135,759	08/04/92	Johnson	424	561	04/26/91
5,346,990	09/13/94	Spaulding	530	350	03/12/91
5,371,585	12/06/94	Morgan et al.	356	246	11/10/92
5,437,987	08/01/95	Ten et al			
5,439,362	08/08/95	Spaulding	424	185.1	07/25/94
5,461,145	10/24/95	Kudo et al			
5,466,572	11/14/95	Sasaki et al.	435	2	04/25/94
5,480,774					
5,483,469	01/09/96	Van den Engh et al.	364	555	08/02/93
5,494,795	2/27/96	Guerri et al.	435	6	5/5/93
5,503,994	04/02/96	Shear et al.	436	90	10/08/93
5,578,449	11/26/96	Frasch et al.	435	6	4/20/95
5,514,537	05/07/96	Chandler	435	002	11/28/94
5,589,457	12/31/96	Wiltbank	514	12	07-03-95
5,602,039	02/11/97	Van den Engh	436	164	10/14/94
5,602,349	02/11/97	Van den Engh	73	864.85	10/14/94
5,622,820	4/11/97	Rossi	435	5	11/3/94
5,641,457	03/09/99	Tomiyama et al.	250	207	06/16/97
5,643,796	07/01/97	Van den Engh et al	436	50	10/14/4
5,660,997	08/26/97	Spaulding	435	7.21	06/07/95
5,690,895	11/25/97	Matsumoto et al.	422	73	12/06/96
5,700,692	12/23/97	Sweet	436	50	09/27/94
5,726,364	03/10/98	Van den Engh	73	864.85	02/10/97
5,819,948	10/13/98	Van den Engh	209	158	08/21/97
5,876,942	3/2/99	Cheng et al	435	6	7/24/97
5,880,457	03/09/99	Tomiyama et al.	250	207	06/16/97
5,985,216	11/16/99	Rens, et al.	422	073	07/24/97
6,071,689	06/06/00	Seidel et al.	435	2	01/29/98

Documentos Extranjeros

DOCUMENTO N°	FECHA	PAIS
WO 96/12171	10/13/95	United States
WO 98/34094	06/08/98	NZ
WO 99/05504	07/24/98	US
WO 99/33956	08/07/99	US
WO 99/38883	05/08/99	US
WO 99/42810	08/26/99	US
WO 00/06193	10/02/00	US

Otros Documentos de Referencia

Roser, JF., Evans, J.W., Kiefer, DP., Neeley, D.P. and Pacheco, C.A. 1980. Reproductive efficiency in mares with anti-hCG antibodies. Proc 9 th Int. Congr. Artira. Repro. and A.I. 4:627. abstr.
"Applying Semen Sexing Technology to the AI Industry", National Association of Animal Breeders, September 2000, pp. 1-16
"Sexed Semen Offers Faster Genetic Gain", Farming News, Livestock Supplement, February 1997, p. 28.
Akhtar, S., et al., "Prevalence of Five Stereotypes of Bluetongue Virus in a Rambouillet Sheep Flock in Pakistan", Veterinary record 136, 1995, p. 495.
Akhtar, S., et al., "Sex Preselected in Cattle: a Field Trial", Veterinary Record 136, 1995, p. 495-496.
Aldrich, S. L., Berger, L.L., Reiling, B.A., Kegler, D.I., and Nagh, T.G.. 1995. "Parturition and periparturient reproductive and metabolic hormone concentration in prenataally androgenized beefheifer", I. Anim. Sci. 73:3712.
Amann, R. P. "Issues affecting commercialization of sexed sperm". Therio: 52:1441, 1999
Amann, R.P. et al, "Prospects For Sexing Mammalian Sperm," Colorado Associated University Press, Animal Reproduction Laboratory College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, 80523, 1982
American Meat Science Association in cooperation with National Livestock and Meat Board. "Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meatK", 1995
Amoah, E.A. and Gelaye, S. 1996. Biotechnological advances in goat reproduction. J. Anim. Sci. 75(2):578-585.
Andersen, V.K., Aamdal, J. and Fougner, J.A. 1973. Intrauterine und tiefzervikale Insemination mit Gefriersperma bein Schat. Zuchthygiene. 8:113-118.
Bagley, C. P. 1993. Nutritional management of replacement beef heifers -A review. J. Anim. Sci. 71:3155-3163.
Bailey, C. M., Reid, C.R., Ringkob, T.P., Koh, Y.O., and Foote, W.D. "Nulliparous versus primiparous crossbred females for beef." J. Anim. Sci. 69:1403., 1991
Baker, R.D., Dziuk, P.J. and Norton, H.W. 1968. Effect of volume of semen, number of sperm and drugs on transport of sperm in artificially inseminated gilts. J. Anim. Sci. 27:88-93.
Barnes, F.L.. and Eyestone, W.H., "Early Cleavage and the Maternal Zygotic Transition in Bovine Embryos", Theriogeneology, Vol. 33, No. 1, January 1990, pp. 141-149
Becker, S.E. and Johnson, A.L. 1992. Effects of gonadotropin releasing hormone infused in a pulsatile or

continuous fashion on serum gonadotropin concentrations and ovulation in the mare. J. Anim. Sci. 70:1208-1215.
Bedford, S. J. and Hinrichs, K. 1994. The effect of insemination volume on pregnancy rates of pony mares. Theriogenology 42:571-578.
Bellows, R. A., Short, R.E., Anderson, D.C., Knapp, B.W., and Pahnish, O.F. "Cause and effect relationships associated with calving difficulty and calfbirth weight", J. Anim. Sci. 33:407, 1971
Berardinelli, J. G., R. A. Dailey, R. L. Butcher, and E. K. Inskeep. "Source of progesterone prior to puberty in beef heifers". J. Anim. Sci. 49:1276., 1979
Berger, G.S. 1987. Intratubal insemination. Fert. Steril. 48:328-330.
Bergfeld, E. G., Kojima, F.N., Cupp, A.S., Wehmann, M.E., Peters, K.T., Garcia-Winder, M., and Kinder, J.E., "Ovarian follicular development in prepubertal heifers is influenced by level of dietary energy-intake", Bio. of Repro. 51:1051, 1994
Berry, B. W., Smith, G.C., and Carpenter, J., "Beef carcass maturity indicators and palatability attributes", J. Anim. Sci. 38:507, 1974
Beyhan, Z., et al., "Sexual Dimorphism in IVF Bovine Embryos Produced by Sperm Sorted by High Speed Flow Cytometry", Theriogenology 49, 1998, p. 359.
Blanchard, T. and Dickson, V., "Stallion Management", The Veterinary Clinics of North America, Equine Practice, Vol. 8, No. 1, April 1992, pp 207 - 218.
Bond, J., et al., "Growth and carcass traits of open beef heifers versus beef heifers that have calved", Nutrition Reports International 34:621. 1986
Boucque, C. V., et al., "Beef-production with maiden and once-calved heifers", Livestock Prod. Sci. 7:121. 1980
Bourdon, R. M. and J. S. Brinks. "Simulated efficiency of range beef -production". Culling strategies and nontraditional management-systems. J. Anim. Sci. 65:963. 1987
Bracher, V. and Allen, W.R., "Videoendoscopic Examination of the Mare's Uterus: Findings in Normal Fertile Mares", Equine Veterinary Journal, Vol. 24 (1992), pp. 274-278
Braselton, W.E. and McShan, W.H. 1970. "Purification and properties of follicle stimulating and luteinizing hormones from horse pituitary glands", Arch. Biochem. Biophys. 139:45-48.
Brethour, J. R., "The single-calfheifer system", Kans. Agric. Sta. Rep. Prog. 570. 1989
Bristol, S.P. 1982. Breeding behavior of a stallion at pasture with 20 mares in synchronized oestrus. J. Reprod. Fert. Suppl. 32:71.
Brookes, A. J. and Obyrne, M., "Use of cow-heifers in beef production", J. of the Royal Agricultural Society of England 126:30. 1965
Buchanan, B.R., et al, "Insemination of Mares with Low Numbers of Either Unsexed or Sexed Spermatozoa", Theriogenology, Vol. 53, pp 1333-1344, (2000)
Burns, P. D. and Spitzer, J.C., "Influence of biostimulation on reproduction in postpartum beef-cows", J. Anim. Sci. 70:358. 1992
Burwash, L.D., Pickett, B.W., Voss, J.L. and Back, D.G. 1974. "Relationship of duration of estrus to pregnancy rate in normally cycling, non-lactating mares" J.A.V.M.A. 165:714-716.
Byerley, D. J., et al., "Pregnancy rates of beef heifers

bred either on puberal or 3rd estrus". J Anim. Sci. 65:645. 1987
Caslick, E.A., "The Vulva and the Vulvo-vaginal Orifice and its Relation to Genital Health of the Thoroughbred Mare", Cornell Veterinarian, Vol. 27, 1937, pp. 178-187
Catt, et al., "Assessment of Ram and Boar Spermatozoa During Cell-Sorting by Flow Cytometry", Reproduction Dom Animal, Vol. 32, 1997, pp 251-258.
Catt, S.L., et al., "Birth of a Male Lamb Derived from an In Vitro Matured Oocyte Fertilized by Intracytoplasmic Injection of a Single Presumptive Male Sperm", Veterinary Record 139, 1996, pp. 494-495.
Chin, W.W. and Boime, I. 1990. In: Glycoprotein Hormones. Serona Symp. Norwell, MA. pp. 19-20
Chung, Y.G., Schenk, J.L., Herickhoff, L.A. and Seidel, G.E. Jr. 1998. Artificial insemination of superovulated heifers with 600,000 sexed sperm. J Anim. Sci. Suppl. 1. 836:215. abstr.
Clement, F., Vincent, P., Mahla, R., Meriaux, J.C. and Palmer, E. 1998. Which insemination fertilizes when several successive inseminations are performed before ovulation. 7 th Int. Symp. Eq. Repro. 151. abstr.
Coleou, J., et al., "Essai de velage tres precoce de genisses en vue de la production de viande." Essai Vauz/Aure no.50, programme USFGC-INAPG-ITFC. 1974
Cran, D.G., et al., "Production of Bovine Calves Following Separation of X- and Y- Chromosome Bearing Sperm and In Vitro Fertilisation", Veterinary Record 132, 1993, pp. 40-41.
Cran, D.G., et al., "Production of Lambs by Low Dose Intrauterine Insemination with Flow Cytometrically Sorted and Unsorted Semen", Theriogenology 47, 1997, p. 267.
Crowley, J. P. The facts of once-bred heifer production. (Ed) J.B. Owens. The maiden female-a means of increasing meat production. School of Agric., Univ. of Aberdeen, Scotland. 1973
Curran, S. 1998. In: Equine Diagnostic Ultrasonography. Fetal gender determination. Rantanen & McKinnon. 1 st Ed. Williams and Wilkins. pp. 165-169.
Day, B.N., Abeydeera, L.R., Johnson, L.A., Welch, G.R., Wang, W.H., Cantley, T.C. and Rieke, A. 1998. Birth of piglets preselected for gender following in vitro fertilization of in vitro matured pig oocytes by X and Y bearing spermatozoa sorted by high speed flow cytometry. Theriogenology. 49(1):360. abstr.
Dean, P.N., Pinkel, D. and Mendelsob. n, M.L. 1978. Hydrodynamic orientation of spermatozoa heads for flow cytometry. Biophys. J. 23:7-13.
Demick, D.S., Voss, J.L. and Pickett, B.W. 1976. Effect of cooling, storage, glycerization and spermatozoal numbers on equine fertility. J. Anim. Sci. 43:633-637.
DenDaas, J.H.G., De Jong, G., Lansbergen, L.M.T.E. and Van Wagtendonk-De Leeuw, A.M. 1998. The relationship between the number of spermatozoa inseminated and the reproductive efficiency of dairy bulls. J Dairy Sci. 81: 1714-1723.
Denham, A. "In-vitro studies on sandhill range forage as related to cattle preference", M.S. Thesis. 1965. Colorado State University.
Deutscher, G. H. "Extending interval from seventeen to nineteen days in the melengestrol acetate-prostaglandin estrous synchronization program for heifers". The Professional Animal Scientist 16:164. 2000
"Diagnostic Products Corporation. Coat-A-Count" ,

Progesterone.com. 1998.
Dikeman, M. E. "Cattle production systems to meet future consumer demands. J. Anim. Sci. 59:1631, 1984
Dinnyes, A., et al., "Timing of the First Cleavage Post-insemination Affects Cryosurvival of In Vitro-produced Bovine Blastocysts", Molec Reprod Develop 53, 1999, pp 318-324.
Donaldson, L. E., "Effect of Insemination Regimen on Embryo Production in Superovulated Cows", The Veterinary Record, July 13, 1985, pp. 35-37
Donoghue, A.M., Byers, A.P., Johnston, L.A., Armstrong, D.L. and Wildt, D.E. 1996. Timing of ovulation after gonadotropin induction and its importance to successful intrauterine insemination in the tiger (<i>Panthera tigris</i>). J. Reprod. Fert. 107:53-58.
Douglas, R.H. 1979. Review of superovulation and embryo transfer in the equine. Theriogenology. 11:33-46.
Douglas, R.H., Nuti, L. and Ginther, O.J. 1974. Induction of ovulation and multiple ovulation on seasonally-anovulatory mares with equine pituitary fractions. Theriogenology. 2(6): 133-142.
Doyle, S. P., et al. "Artificial insemination of lactating angus cows with sexed semen". Proc. Western Sect. Am.Soc.Anim. Sci. 50:203. 1999
Duchamp, G., Bour, B., Combamous, Y. and Palmer, E. 1987. Alternative solutions to hCG induction of ovulation in the mare. J. Reprod. Fert. Suppl. 35:221-228.
Evans, M.J. and Irvine, C.H.G. 1977. Induction of follicular development, maturation and ovulation by gonadotropin releasing hormone administration to acyclic mares. Bio. Reprod. 16:452-462.
Ferrell, C. L. and T. G. Jenkins. "Energy-Utilization by Mature, nonpregnant, nonlactating cows of different types" J. Anim. Sci. 58:234. 1984
Ferrell, C. L. "Effects of post-weaning rate of gain on onset of puberty and productive performance of heifers of different breeds. J. Anim. Sci. 55:1272. 1982
Field, R. A., et al., "Bone-ossification and carcass characteristics of wethers given silastic implants containing estradiol". I. Anim. Sci. 68:3663-3668. 1990
Field, R., R. et al., "Growth, carcass, and tenderness characteristics of virgin, spayed, and single-calf heifers.", J. Anim. Sci. 74:2178. 1996
Fitzgerald, B.P., Peterson, K.D. and Silvia, P.J. 1993. Effect of constant administration of a gonadotropin-releasing hormone agonist on reproductive activity in mares: Preliminary evidence on suppression of ovulation during the breeding season. Am. J. Vet. Res. 54:1746-1751.
Fluharty, F. L., et al., "Effect of weaning and diet on growth of calves." Research and Reviews. The Ohio State University Department of Animal Sciences. 1996
Fluharty, F.L., et al., "Effects of Age at Weaning and Diet on Growth of Calves", Ohio Agri. Res. and Dev. Circular, 1996, 156: 29.
Foulkes, J.A., Stewart, D.L. and Herbert, C.N. 1977. Artificial insemination of cattle using varying numbers of spermatozoa. Vet. Rec. 101:205.
Fugger, E.F., "Clinical Experience with Flow Cytometric Separation of Human X- and Y- Chromosome Bearing Sperm", Theriogenology, Vol. 52, pp. 1435-1440 (1999)
Fulwyler, M.J. 1965. Electronic separation of biological cells by volume. Science. 150:910.
Fulwyler, M.J. 1977. Hydrodynamic orientation of cells. J

Histochem. Cytochem. 25:781-783.
Seidel, G.E.. Jr., "Artificial Insemination With X-and Y-Bearing Bovine Sperm", Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO; Germplasm and Gamete Physiology Lab, ARS, USDA, Beltsville, MD; Atlantic Breeders Coop, Lancaster, PA; DUO Diary, Loveland, CO, USA January 1996.
Garner, D.L., Gledhill, B.L., Pinkel, D., Lake, S., Stephenson, D., Van Dilla, M.A. and Johnson, L.A. 1983. "Quantification of the X and Y chromosome-bearing spermatozoa of domestic animals by flow cytometry". Biol. Reprod. 28:312-321.
Ginther, O.J. 1983. Sexual behavior following introduction of a stallion into a group of mares. Theriogenology. 19:877.
Ginther, O.J. 1992. In: Reproductive Biology of the Mare. (2nd Ed.) Equiservices, Cross Plains, WI.
Gledhill, B.L. 1988. Gender preselection: historical, technical and ethical perspective. Semin Reprod. Endocrinol. 6:385-395.
Gombe, S. and Hansel, W. "Plasma luteinizing-hormone (LH) and progesterone levels in heifers on restricted energy intakes." J. Anim. Sci. 37:728. 1973
Gourley, D.D. and Riese, R.L. 1990. Laparoscopic artificial insemination in sheep. Vet. Clin. N. Amer: Food Anim. Prac. 6(3):615-633.
Gravert, H. O., "Genetic Aspects of Early Calving." In: J.C. Taylor (Ed.) The early calving of heifers and it's impact on beef production. 59. 1975
Gregory, K. E., et al., "Characterization of biological types of cattle III .2." Growth-rate and puberty in females. J. Anim. Sci. 49:461. 1979
Grimes, I. F, and T. B. Turner. "Early weaning of fall born calves II." Post weaning performance of early and normal-weaned calves. I. Prod. Agric. 4:168. 1991
Grondahl, C., et al, "In Vitro Production of Equine Embryos", Biology of Reproduction, Monograph Series I, pp. 299-307 (1995)
Guillou, F. and Combamous, Y. 1983. Purification of equine gonadotropins and comparative study of their acid-dissociation and receptor-binding specificity. Biochem. Biophys. Acta. 755:229-236.
Gurnsey, M.P., and Johnson, L.A., "Recent improvements in efficiency of flow cytometric sorting of X and Y-chromosome bearing sperm of domestic animals: a review", 1998, New Zealand Society of Animal Protection, three pages.
Hall, J. B., et al., "Effect of age and pattern of gain on induction of puberty with a progestin in beef heifers." J. Anim. Sci. 75:1606. 1997
Hamano, K., et al., "Gender Preselection in Cattle with Intracytoplasmically Injected, Flow Cytometrically Sorted Sperm Heads", biology of Reproduction 60, 1999, pp. 1194-1197.
Harrison, L.A., Squires, E.L. and McKinnon, A.O. 1991. Comparison of hCG, buserelin and luprostitol for induction of ovulation in cycling mares. Eq. Vet. Sci. 3:163-166.
Harte, F. J. "System of production of bee from once calved heifers." In: J.C. Taylor (Ed.) The early calving of heifers and it's impact on beef production. 123. 1975
Hawk, H.W., et al., "Fertilization Rates in Superovulating Cows After Deposition of Semen on the Infundibulum Near the Uterotubal Junction or After Insemination with High Numbers of Sperm", XP-002103478,

Theriogenology, May 1988, Vol. 29, No. 5, pp 1131-1142.
Hemlesmeyer, G. N., et al. "Effects of lactation and prenatal androgenization on the performance, carcass composition and longissimus muscle sensory characteristics of heifers in the single-calf heifer system." The Professional Animal Scientist 15:14. 1999
Hennegmeyer, G. N., et al. "Effects of prenatal androgenization and implantation on the performance and carcass composition of lactating heifers in the single-calf heifer system." The Professional Animal Scientist 15:173. 1999
Hilton, G. G., et al., "An evaluation of current and alternative systems for quality grading carcasses of mature slaughter cows." I. Anim. Sci. 76:2094. 1998
Ho, L., et al., "Influence of gender, breed and age on maturity characteristics of sheep." J. Anim. Sci. 67:2460-2470. 1989
Hofferer, S., Lecompte, F., Magallon, T., Palmer, E. and Combamous, Y. 1993. Induction of ovulation and superovulation in mares using equine LH and FSH separated by hydrophobic interaction chromatography. J. Reprod. Fert. 98:597-602.
Hohenboken, W. D. "Applications of sexed semen in cattle production." Therio. 52:1421. 1999
Holtan, D.W., Douglas, R.H. and Ginther, O.J. 1977. Estrus, ovulation and conception following synchronization with progesterone, prostaglandin F2 ct and human chorionic gonadotropin in pony mares. J. Anim. Sci. 44:431-437.
Householder, D.D., Pickett, B.W., Voss, J.L. and Olar, T.T. 1981. Effect of extender, number of spermatozoa and hCG on equine fertility. J. Equine Vet. Sci. 1:9-13.
Howard, J.G., Bush, M., Morton, C., Morton, F., Wentzel, K. and Wildt, D.E. 1991. Comparative semen cryopreservation in ferrets (<i>Mustela putorius furo</i>) and pregnancies after laparoscopic intrauterine insemination with frozen-thawed spermatozoa. J. Reprod. Fert. 92:109-118.
Howard, J.G., Roth, T.L., Byers, A.P., Swanson, W.F. and Wildt, D.E. 1997. Sensivity to exogenous gonadotropins for ovulation and laparoscopic artificial insemination in the theetab and clouded leopard. Biol. Reprod. 56:1059-1068.
Hunter, R.H.F. 1980. Transport and storage of spermatozoa in the female reproductive tract. Proc 4 th Int. Congr. Artira. Repro. and A.I. 9:227-233.
Hyland, J.H., Ainsworth, C.G.V. and Langsford, D.A. 1988. Gonadotropin-releasing hormone (GnRH) delivered by continuous infusion induces fertile estrus in mares during seasonal acyclicity. Proc. Amer. Assoc. Eq. Prac. 181-190.
Irvine, C.H.G. and Alexander, S.L. 1993. In: Equine Reproduction. Edited by McKirmon and Voss. Lea and Febiger. Philadelphia, London. pp. 37.
Jafar, et al., "Sex Selection in Mammals: A Review", Theriogenology, vol. 46, 1996, pp 191-200.
Jarriage, R. "Age of cows at first calving in France." J.C. Taylor (Ed.) The early calving of heifers and it's impact on beef production. 10. 1975
Jasko, D.J., Martin, J.M. and Squires, E.L. 1992. Effect of volume and concentration of spermatozoa on embryo recovery in mares. Theriogenology. 37:1233-1239
Johnson L.A., et al., 1987. Flow cytometry of X- and Y-chromosome bearing sperm for DNA using an improved

preparation method and staining with Hoechst 333-42. <i>Garnete Research</i> 17: 203-212
Johnson, "Gender preselection in Mammals: An overview", <i>Dtsch. Tierarztl. Wschr</i> , Vol. 103, Aug./Sep. 1996, pp 288-291.
Johnson, A.L. 1986. Pulsatile release of gonadotropin releasing hormone advances ovulation in cycling mares. <i>Biol. Reprod.</i> 35:1123E 1130.
Johnson, A.L. and Becker, S.E. 1988. Use of gonadotropin-releasing hormone (GnRH) treatment to induce multiple ovulations in the anestrous mare. <i>Eq. Vet. Sci.</i> 8:130-134.
Johnson, L., "Sex Preselection by Flow Cytometric Separation of X and Y Chromosome-Bearing Sperm Based on DNA Difference: a Review", <i>Reproduction and Fertilization Development</i> 7, 1995, pp. 893-903.
Johnson, L., "Successful Gender Preselection in Farm Animals", <i>Agricultural Biotechnology</i> , 1998, pp. 439-452.
Johnson, L.A. 1988. Flow cytometric determination of spermatozoa sex ratio in semen purportedly enriched for X or Y bearing spermatozoa. <i>Theriogenology</i> . 29:265. abstr.
Johnson, L.A. 1992. Gender preselection in domestic animals using flow cytometrically sorted sperm. <i>J Anim. Sci. Suppl</i> 1.70:8-18.
Johnson, L.A. 1994. Isolation of X- and Y-bearing spermatozoa for sex preselection. In: <i>Oxford Reviews of Reproductive Biology</i> . Ed. HH Charlton. Oxford University Press. 303-326.
Johnson, L.A. 1995. Sex preselection by flow cytometric separation of X and Y chromosome bearing spermatozoa based on DNA difference: a review. <i>Reprod. Fert. Dev.</i> 7:893-903.
Johnson, L.A. and Schulman, J.D. 1994. The safety of sperm selection by flow cytometry. <i>Ham. Reprod.</i> 9(5):758.
Johnson, L.A., "Sex preselection in porcinos: altered sex ratios in offspring following surgical insemination of flow-sorted X- and Y-bearing sperm", <i>Reprod. Domest. Anim.</i> 26:309-314, 1991
Johnson, L.A., and Pinkel, D., "Modification of a Laser-Based flow Cytometer for High-Resolution DNA Analysis of Mammalian Spermatozoa", <i>Cytometry</i> 7, 1986, pp 268 - 273.
Johnson, L.A., et al., "Sex Preselection in Rabbits: Live Births from X and Y Sperm Separated by DNA and Cell Sorting", <i>Exceptional Paper-Rapid Publication</i> , XP-002103476, <i>Biology of Reproduction</i> 41, 199-203, 1989, pp 199-203.
Johnson, L.A., et al., 1994. Improved flow sorting resolution of X- and Y- chromosome bearing viable sperm separation using dual staining and dead cell gating. <i>Cytometry</i> 17 (suppl 7):83.
Johnson, L.A., Flook, J.P., Look, M.V. and Pinkel, D. 1987b. Flow sorting of X and Y chromosome bearing spermatozoa into two populations. <i>Gam. Res.</i> 16:203-212.
Johnson, L.A., Welch, G.R., Rens, W. and Dobrinsky, J.R. 1998. Enhanced flow cytometric sorting of mammalian X and Y sperm: high speed sorting and orienting no77.1e for artificial insemination. <i>Theriogenology</i> . 49(1):361. abstr.
Joseph, R. L. "Carcass composition and meat quality in once calved heifers." In: J.C. Taylor (Ed.) <i>The early calving of heifers and it's impact on beef production</i> . 143. 1975
Joseph, R. L. and J. P. Crowley. "Meat quality of once-calved heifers." <i>Irish J. of Agric. Research</i> 10:281.

1971
Kachel, V., et al., AUniform Lateral Orientation, Cused by Flow Forces, of Flat Particles in Flow-Through Systems®, The Journal of Histochemistry and Cytochemistry, 1997, Vol. 25, No. 7, pp 774 -780.
Kanayama, K., Sankai, T., Nariaik, K., Endo, T. and Sakuma, Y. 1992b. Pregnancy by means of tubal insemination and subsequent spontaneous pregnancy in rabbits. J. Int. Med. Res. 20:401-405.
Karabinus, et al., "Effects of Egg Yolk-Citrate and Milk Extenders on Chromatin Structured Viability of Cryopreserved Bull Sperm", Journal of Dairy Science, Vol. 74, No. 11, 1999, pp 3836-3848.
Keeling, P. C. B. M. S. T. G. D. I. a. P. W. J., "A modeling study of once-bred heifer beef production." Proceedings of the New Zealand Society of Animal Production. 51. 1991
Kilicarslan, M.R., Horoz, H., Senunver, S.C., Konuk, S.C., Tek, C. and Carioglu, B. 1996. Effect of GrnRH and hCG on ovulation and pregnancy in mares. Vet. Rec. 139:119-120.
Kinder, J. E., et al. "Endocrine basis for puberty in heifers and ewes." J. Repro. and Fertility 393. 1995
Klindt, J. and J. D. Crouse. "Effect of ovariectomy and ovariectomy with ovarian auto transplantation on feedlot performance and carcass characteristics of heifers." J. Anim. Sci. 68:3481. 1990
Klosterman, E. W. and C. F. Parker. "Effect of size, breed and sex upon feed efficiency in beef cattle." North Central Regional Research Publication 235, Ohio Agric. Research and Development Center 1090:3. 1976
Kniffen, D. M., Wagner, W.R., and Lewis. P.E. "Effects of long-term estrogen implants in beef heifers." I. Anim. Sci. 77:2886. 1999
Koch, R. M., et al., "Characterization of biological types of cattle -Cycle-II .3." Carcass composition, quality and palatability. I. Anim. Sci. 49:448. 1919
Lapin, D.R. and Ginther, O.J. 1977. Induction of ovulation and multiple ovulations in seasonally anovulatory and ovulatory mares with an equine pituitary extract. J. Anim. Sci. 44:834-842.
Laster, D. B., "Factors affecting dystocia and effects of dystocia on subsequent reproduction in beef-cattle." J. Anim. Sci. 36:695. 1973
Lawrenz, R. 1985. Preliminary results of non-surgical intrauterine insemination of sheep with thawed frozen semen. J S Afr. Vet. Assoc. 56(2):61-63.
Levinson, G., et al, 1995. DNA-based X-enriched sperm separation as an adjunct to preimplantation genetic testing for the preparation of X-linked disease. Mol. Human Reprod. 10:979-982.
Lindsey, A., et al., AHysteroscopic Insemination of Mares with Nonfrozen Low-dose Unsexed or Sex-sorted Spermatozoa®, currently unpublished, pp. 1-15.
Linge, F. 1972. Faltforsok med djupfrost sperma (field trials with frozen sperm). Farskotsel. 52:12-13.
Lonergan, P., et al., "Effect of Time Interval from Insemination to First Cleavage on the Development of Bovine Embryos In Vitro and In Vivo", Theriogenology, 1999, p. 326
Long, C.R., Rath, D., Welch, G.R., Schreier, L.L., Dobrinsky, J.R. and Johnson, L.A. 1998. AIn vitro production of porcine embryos from semen sorted for sex with a high speed cell sorter: comparison of two

fertilization media.®, Theriogenology. 49(1):363. abstr.
Loy, R.G. and Hughes, J.P. 1965. The effects of human chorionic gonadotropin on ovulation, length of estrus, and fertility in the mare. Cornell Vet. 56:41-50.
Lu, K.H., et al., "In Vitro Fertilization with Flow-Cytometrically-Sorted Bovine Sperm", Theriogenology 52, 1999, pp. 1393-1405.
Lynch, I. M., et al., "Influence of timing of gain on growth and reproductive performance of beef replacement heifers." I. Anim. Sci. 75:1715. 1997
Macmillan, K.L. and A.M. Day, "Prostaglandin F2a - A Fertility Drug In Dairy Cattle?", Ruakura Animal Research Station, Private Bag, Hamilton, New Zealand, Theriogenology, September 1982, Vol. 18 No. 3, pages 245-253
Martin, A. H., et al., "Characteristics of youthful beef carcasses in relation to weight, age and sex .3. meat quality attributes." Canadian I. Anim. Sci. 51:305. 1971
Martin, L. C., J. S. Brinks, R. M. Bourdon, and L. V. Cundiff. "Genetic-effects on beef heifer puberty and subsequent reproduction." J. Anim. Sci. 70:4006. 1992
Matsuda, Y. and Tobari, I. 1988. Chromosomal analysis in mouse eggs fertilized in vitro with sperm exposed to ultraviolet light (UV) and methyl and ethyl methanesulfonate (MMS and EMS). Mutat. Res. 198:131-144.
Matulis, R. J., F. K. McKeith, D. B. Faulkner, L. L. Berger, and P. George. "Growth and carcass characteristics of cull cows after different times-on-feed." J. Anim. Sci. 65:669. 1987
Mauleon, P. "Recent research related to the physiology of puberty." Commission of the European Communities. The early calving of heifers and it's impact on beef production. 1975
Maxwell, W. and Johnson, L., "Chlortetracycline Analysis of Boar Spermatozoa after Incubation, Flow Cytometric Sorting, Cooling, or Cryopreservation", Molecular Reproduction and Development 46, 1997, pp. 408-418.
Maxwell, W.M.C., Evans, G., Rhodes, S.L., Hillard, M.A. and Bindon, B.M. 1993. Fertility of Superovulated Ewes after Intrauterine or Oviductal Insemination with Low Numbers of Fresh or Frozen-Thawed Spermatozoa. Reprod. Fertil. Dev. 5:57-63.
Mccomlick, R. J. "The flexibility of the collagen compartment of muscle." Meat Sci. 36:79. 1994
McCue, P.M. 1996. Superovulation. Vet. Clin. N. Amer. Eq. Prac. 12:1-11.
McCue, P.M., Fleury, J.J., Denniston, D.J., Graham, J.K. and Squires, E.L. 1997. Oviductal insemination in the mare. 7 th Int Symp. Eq. Reprod. 133. abstr.
McDonald, L.E. 1988. Hormones of the pituitary gland. In: Veterinary Pharmacology and Therapeutics. 6 th ed. Edited by N.H. Booth and L.E. McDonald. Ames, Iowa State Univ. Press. pp. 590.
McKenna, T., Lenz, R.W., Fenton, S.E. and Ax, R.L. 1990. Nonreturn rates of dairy cattle following uterine body or comual insemination. J. Dairy Sci. 73:1179-1783.
McKinnin, A. and Voss, J., "Equine Reproduction", Lea & Febiger, Philadelphia, 1993, pp 291, 299 - 302, 345 - 348, 739 - 797.
McKinnon, A. et al, 1993. Predictable ovulation in mares treated with an implant of the GnRH analogue deslorelin. Eq. Vet. J. 25:321-323.
McKinnon, A.O. et al, 1996. Repeated use of a GnRH analogue deslorelin (Ovuplant) for hastening ovulation in

the transitional mare. <i>Eq. Vet. J.</i> 29:153-155.
McNutt, et al., "Flow Cytometric Sorting of Sperm: Influence on Fertilization and Embryo/Fetal Development in the Rabbits", <i>Molecular Reproduction and Development</i> , Vol. 43, 1996, pp 261-267.
Meilgaard, M., G. V. Civile, and B. T. Carr. "Sensor Evaluation Techniques." CRC Press Inc., Boca Raton, FL. 1991
Meinert, C., et al., "Advancing the time of ovulation in the mare with a short-term implant releasing the GnRH analogue deslorelin", <i>Equine Veterinary Journal</i> , 25, 1993, pp 65 - 68.
Merton, J., et al., "Effect of Flow Cytometrically Sorted Frozen/Thawed Semen on Success Rate of In Vitro Bovine Embryo Production", <i>Theriogenology</i> 47, 1997, pp. 295.
Meyers, P.J., Bowman, T., Blodgett, G., Conboy, H.S., Gimenez, T., Reid, M.P., Taylor, B.C., Thayer, J., Jochle, W. and Trigg, T.E. 1997. Use of the GnRH analogue, deslorelin acetate, in a slow release implant to accelerate ovulation in oestrous mares. <i>Vet. Rec.</i> 140:249-252.
Michaels, Charles, "Beef A.I. Facilities that work", <i>Proc. Fifth N.A.A.B Tech. Conf. A.I. Reprod. Columbia, MO.</i> pp. 20-22.
Michel, T.H., Rossdale, P.D. and Cash, R.S.G. 1986. Efficacy of human chorionic gonadotrophin and gonadatrophin releasing hormone for hastening ovulation in Thoroughbred mares. <i>Eq. Vet. J.</i> 6:438-442.
Miller, S.J. 1986. <i>Artificial Breeding Techniques in Sheep</i> . In Morrow, D.A. (ed): <i>Current Therapy in Theriogenology</i> 2. Philadelphia, WB Saunders.
Mirskaja, L.M. and Petrapavlovskii, V.V. 1937. The reproduction of normal duration of heat in the mare by the administration of Prolan. <i>Probl. Zivotn. Anim. Breed. Abstr.</i> 5:387.
Moe, P. W., H. F. Tyrrell, and W. P. Flatt. "Energetics of body tissue mobilization." <i>J. of Dairy Sci.</i> 54:548.
Molinia, F.C., Gibson, R.J., Brown, A.M., Glazier, A.M. and Rodger, J.C. 1998. Successful fertilization after superovulation and laparoscopic intrauterine insemination of the brushtail possum, <i>Trichosurus vulpecula</i> , and tammar wallaby, <i>Macropus eugenii</i> . <i>J.Reprod. Fert.</i> 112:9-17.
Moms, S. T., et al., "Biological efficiency: How relevant is this concept to beef cows in a mixed livestock seasonal pasture supply context?" <i>Proceedings of the New Zealand Society of Animal Production</i> 54:333. 1994
Monensin." <i>J. Anim. Sci.</i> 55:357-362. 1982
Moran, C., J. F. Quirke, and J. F. Roche. "Puberty in heifers -a review." <i>Animal Reproduction Sci.</i> 18:167. 1989
Morcom, C.B. and Dukelow, W.R. 1980. A research technique for the oviductal insemination of pigs using laparoscopy. <i>Lab. Anim. Sci.</i> 1030-1031.
Morgan, J. B., et al., "National beef tenderness survey." <i>J. Anim. Sci.</i> 69:3274. 1991
Morris, L.H., et al., "Hysteroscopic insemination of small numbers of spermatozoa at the uterotubal junction of preovulatory mares", <i>Journal of Reproduction and Fertility</i> , Vol. 118, pp. 95-100 (2000)
Moseley, W. M., et al., 1982. "Relationship of Growth and Puberty in Beef Heifers Fed
Mount, D. E. "Fibrous and non-fibrous carbohydrate supplementation to ruminants grazing forage from small grain crops." M.S. Thesis. Colorado State University.

2000
Muller, W. and Gautier, F. 1975. Interactions of heteroaromatic compounds with nucleic acids. <i>Euro. J Biochem.</i> 54:358.
Munne, S. 1994. Flow cytometry separation of X and Y spermatozoa could be detrimental to human embryos. <i>Hum. Reprod.</i> 9(5):758
Myers, S. E., "Performance and carcass traits of early-weaned steers receiving either a pasture growing period or a finishing diet at weaning." <i>J. Anim. Sci.</i> 77:311. 1999
Myers, S. E., et al., "Comparison of three weaning ages on cow-calf performance and steer carcass traits." <i>J. Anim. Sci.</i> 77:323. 1999
Myers, S. E., et al., "Production systems comparing early weaning to normal weaning with or without creep feeding for beef steers." <i>J. Anim. Sci.</i> 77:300. 1999
Nix, I. P., I. C. Spitzer, and P. I. Chenoweth. "Serum testosterone concentration, efficiency of estrus detection and libido expression in androgenized beef cows." <i>Therio.</i> 49: 1195. 1998
Nowshari, et al., "Superovulation of Goats with Purified pFSH Supplemented with Defined Amounts of pLH", <i>Theriogenology</i> , Vol 43, 1995, pp 797-802.
Nowshari, et al., <i>Theriogenology</i> , Vol 43, 1995, pp 797-802.
NRC. Nutrient requirements for beef cattle. National Academy of Sci. National Research Council, Washington, DC. 1996
Olson, S.E. and Seidel, G.E. Jr., "Reduced Oxygen Tension and EDTA improve Bovine Zygote Development in a Chemically Defined Medium", <i>Journal of Animal Science</i> 78, 2000, pp. 152-157.
Owen, J. B. "The maiden female-a means of increasing meat production." <i>Proc. Symp. on the use of once bred heifers and gilts.</i> 1973
Pace, M.M. and Sullivan, J.J. 1975. Effect of timing of insemination, numbers of spermatozoa and extender components on pregnancy rates in mares inseminated with frozen stallion semen. <i>J Reprod. Fert. Suppl.</i> 23:115-121.
Parent US Application 09/001,394, entitled "Sheath Fluids and Collection Systems for Sex-Specific Cytometer Sorting of Sperm", filed on December 31, 1997, 87 total pages which includes four drawings.
Parrish, J., et al., "Capacitation of Bovine Sperm by Heparin", <i>Technology of Reproduction</i> 38, 1988, pp. 1171-1180.
PCT application, PCT/US99/17165, filed 28 July 1999, entitled "Equine System for Non-Surgical Artificial Insemination".
PCT application, PCT/US98/27909, filed 31 December 1998, entitled "Commercially Practical Sex-Specific Insemination of Mammals".
Peippo, J., et al., "Sex diagnosis of equine preimplantation embryos using the polymerase chain reaction", <i>Theriogenology</i> , Vol. 44 619-627 (1995)
Perry, E.J. 1968. Historical Background In: <i>The Artificial Insemination of Farm Animals</i> . 4th ed. Edited by E.J. Perry. New Brunswick, Rutgers University Press, pp. 3-12.
Petersen, G.A., et al, "Cow and Calf Performance and Economic Considerations of Early Weaning of Fall-Born Beef Calves", <i>J. Anim. Sci.</i> , 1987, 64:15, pp 15-22.
Petit, M. "Early Calving in Suckling Herds." In: (Ed.)

J.C. Taylor. The early calving of heifers and it's impact on beef production. 157. 1975
Pickett GW, et al., "Management of the mare for maximum reproductive efficiency", Bulletin No. 6 Colorado State University, Ft. Collins CO. (1989)
Pickett, B.W, et al., 1976. Factors influencing the fertility of stallion spermatozoa in an A.I. program. Proc. 8 th Internat. Congr. Anim. Reprod. A.I. Krakow, Poland. 4: 1049 - 1052.
Pickett, B.W. and Back, D.G. 1973. Procedures for preparation, collection, evaluation and insemination of stallion semen. C.S.U. Exp. Sta. Artira. Reprod. Lab. Gen. Series Bull. 935.
Pickett, B.W., and Shiner, K.A., "Recent developments in artificial insemination in horses", Livestock Production Science, 40, 1994, pp 31 - 36.
Pickett, B.W., Burwash, L.D., Voss, J.L. and Back, D.G. 1975b. Effect of seminal extenders on equine fertility. J. Anim. Sci. 40:1136-1143.
Pinkel, D., et al, "Flow Cytometric Determination of the Proportions of X- and Y- Chromosome-Bearing Sperm in Samples of Purportedly Separated Bull Sperm", Journal of Animal Science, Vol. 60, No. 5, 1985, pp 1303 - 1307.
Pinkel, D., Gledhill, B.L., Van Dilla, M.A., Stephenson, D. and Watchmaker, G. 1982b. High resolution DNA measurements of mammalian spermatozoa. Cytometry. 3:1-9. (1982b)
Polge, E. J., "Historical Perspective of AI: Commercial Methods of Producing Sex Specific Semen, IVF Procedures", Proceedings of the 16 th Technical Conference on Artificial Insemination & Reproduction, Cambridge, England, 1996, pp. 7-11.
Purvis, H. T. and J. C. Whittier. "Effects of ionophore feeding and anthelmintic administration on age and weight at puberty in spring-born beef heifers." J. Anim. Sci. 74:736-744. 1996
Randel, R. D. "Nutrition and postpartum rebreeding in cattle." J. Anim. Sci. 68:853. 1990
Rath, D., et al., "Low Dose Insemination Technique in the Pig", Boar Semen Preservation IV, 2000, pp. 115-118.
Rath, D., et al., "Production of Piglets Preselected for Sex Following in Vitro Fertilization with X and Y Chromosome-Bearing Spermatozoa Sorted by Flow Cytometry", Theriogenology, 47, 1997, pp 795 - 800.
Reiling, B.A., et al., "Effect of Prenatal Androgenization on Performance, Location, and Carcass and Sensory Traits on Heifers in Single Calf Heifer System", J. Anim. Sci., 1995, 73: 986, pp 986-992.
Rens, W., et al, "A Novel Nozzle for More Efficient Sperm Orientation to Improve Sorting Efficiency of X and Y Chromosome-Bearing Sperm", Cytometry 33, 1998, pp. 476-481
Rens, W., et al., "Improved Flow Cytometric Sorting of X- and Y- Chromosome Bearing Sperm: Substantial Increase in Yield of Sexed Semen", Molecular Reproduction and Development, 1999, pp 50-56.
Rieger, D., et al, "The Relationship Between the Time of First Cleavage of Fertilized Cattle Oocytes and Their Development to the Blastocyst Stage", Theriogenology, 1999, pp. 190.
Ritar, A. and Ball, A. 1991. Fertility of young cashmere goats after laparoscopic insemination. J. Agr. Sci. 117:271-273.
Roberts, J.R. 1971. In: Veterinary Obstetrics and Genital

<i>Diseases</i> . Ithaca, New York. pp. 740-749.
Romita, A. "Some considerations on the beef situation in Italy." (Ed.) J.C. Taylor. <i>The early calving of heifers and it's impact on beef production</i> . 23. 1975
Roth, T.L., Wolfe, B.A., Long, J.A., Howard, J. and Wildt, D.E. 1997. Effects of equine chorionic gonadotropin, human chorionic gonadotropin, and laparoscopic artificial insemination on embryo, endocrine, and luteal characteristics in the domestic cat. <i>Bio Reprod</i> . 57:165-171.
Roux, M., J. H. Teissier, J. Bonnemaire, and R. Dumont. "Early calving heifers versus maiden heifers for beef -production from dairy herds. 1." The effects of genotype (Friesian and Charolais x Friesian) and 2 feeding levels in the rearing period on growth and carcass quality. <i>Livestock Prod. Sci</i> .16:1. 1987
Rowley, H-S., Squires, E.L. and Pickett, B.W. 1990. Effect ofinsemination volume on embryo recover}' in mares. <i>J. Equine Vet. Sci</i> . 10:298-300.
Roy, J. H. B. "Rearing dairy-herd replacements." <i>J. of the Soc. ofDairy Technology</i> 31:73-79. 1978
Rutter, L. M., et al., "Effect of abomasal infusion of propionate on the GnRH-induced luteinizing-hormone release in prepuberal heifers." <i>J. Anim. Sci</i> . 56:1167. 1983
Salamon, S. 1976. <i>Artificial Insemination of Sheep</i> . Chippendale, New South Wales. Publicity Press. p.83-84.
Salisbury, G.W. and VanDemark, N.L. 1961. <i>Physiology of Reproduction and Artificial Insemination of Cattle</i> . San Francisco: Freeman and Company.
SAS, SAS/STAT, "Useres Guide (Release 6.03)", SAS Inst. Inc., Cary, NC., 1988. 3 pages
SAS. "The SAS System for Windows." Ver 7.0. ReI 6.12. SAS Inst.lnc., Cary, NC. 2000
Schenk, J. L., T. K. Suh, D. G. Cran, and G. E. Seidel. "Cryopreservation of flow-sorted bovine spennatozoa." <i>Therio</i> . 52:1375. 1999
Schenk, J.L. and Seidel, Jr., G.E., "Imminent Commercialization of Sexed Bovine", <i>Proceedings, The Range Beef Cow Symposium XVI</i> , 1999, pp 89-96.
Schillo, K. K., J. B. Hall, and S. M. Hileman. "Effects of nutrition and season on the onset of puberty in the beef heifer." <i>J. Anim. Sci</i> . 70:3994. 1992
Schmid R.L., et al, "Fertilization with Sexed Equine Spermatozoa Using Intracytoplasmic Sperm Injection and Oviductal Insemination", 7th International Symposium On Equine Reproduction, pp. 139 (Abstract) (1998)
Schnell, T. D., K. E. Belk, J. D. Tatum, R. K. Miller, and G. C. Smith. "Performance, carcass, and palatability traits for cull cows fed high-energy concentrate diets for 0, 14,28,42, or 56 days." <i>J. Anim. Sci</i> . 75:1195. 1997
Schoonmaker, J. P., et al., "Effects of age at weaning and implant strategy on growth of steer calves." <i>J. Anim. Sci. (SuppI2)</i> 76:71 (Abstr.). 1998
Seidel, G. E. and L. A. Johnson. "Sexing mammalian spenn -overview." <i>Therio</i> . 52: 1267. 1999
Seidel, G. E., "Insemination of heifers with sexed sperm." <i>Therio</i> . 52:1407. 1999
Seidel, G.E. Jr., "Uterine Horn Insemination of Heifers With Very Low Numbers of Nonfrozen and Sexed Spermatozoa", <i>Atlantic Breeders Cooperative, Theriogenology</i> 48: pp. 1255-1264, (1997)
Seidel, G.E. Jr., Cran, D.G., Herickoff, L.A., Schenk, J.L., Doyle, S.P. and Green, R.D. 1999. Insemination of

heifers with sexed frozen or sexed liquid semen. Theriogenology. 51. (in press). abstr.(1999)
Seidel, G.E., Jr., et al, "Artificial Insemination With X-and Y-Bearing Bovine Sperm", Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO; Germplasm and Gamete Physiology Lab, ARS, USDA, Beltsville, MD; Atlantic Breeders Coop, Lancaster, PA; DUO Dairy, Loveland, CO, USA January 1996.
Seidel, G.E., Jr., et al, "Insemination Of Heifers With Very Low Numbers Of Frozen Spermatozoa." , Colorado State University, Fort Collins, Atlantic Breeders Cooperative, Lancaster, PA, DUO Dairy, Loveland, CO, July 1996.
Seidel, Jr., G. E., et al, "Insemination of Holstein Heifers With Very Low Numbers Of Unfrozen Spermatozoa", Colorado State University, Atlantic Breeders Cooperative, (1995)
Seidel, Jr., G.E.et al, "Insemination Of Heifers With Very Low Numbers Of Frozen Spermatozoa", Colorado State University (1996)
Sell, R. S., D. L. Watt, R. D. Little, and T. A. Petry. "Single-calfheifer profitability compared to other north dakota beef production systems." Department of Ag. Eco., North Dakota State University, Ag. Econ. Rpt. 20.
Senger, P.L., Becker, W.C., Davidge, S.T., Hillers, J.K. and Reeves, J.J. 1988. Influence of comual insemination on conception rates in dairy cattle. J Anim. Sci. 66:3010-3016.
Shackelford, S. D., M. Koohmaraie, and T. L. Wheeler. "Effects of slaughter age on meat tenderness and usda carcass maturity scores of beef females." I. Anim. Sci. 73:3304. 1995
Shelton, J.N. and Moore, N.W. 1967. The response of the ewe tot pregnant mare gonadotropin and to horse anterior pituitary extract. J. Reprod. Fert. 14:175 - 177.
Shilova, A.V., Platov, E.M. and Lebedev, S.G. 1976. The use of human chorionic gonadotrophin for ovulation date regulation in mares. VIIITH Int. Congr. On Anim. Repro. and A.I. 204-208.
Shorthose, W. R. and P. V. Harris. "Effect of animal age on the tenderness of selected beef muscles." I. Food Sci. 55:1-. 1990
Silbennann, M., "Honnonos and Cartilage. Cartilage: development, differentiation, and growth." pp. 327-368. Academic Press, Inc. 1983
Simon, M., "The effect of management option on the perfonnance of pregnant feedlot heifers." M.S. Thesis. Kansas State University. 1983
Smith, G. C., B. W. Berry, J. W. Savell, and H. R. Cross. "USDA maturity indexes and palatability ofbeefrib steaks." J. of Food Quality 11 :1. 1988
Smith, G. C., et al., "Relationship ofusda maturity groups to palatability of cooked beef." J. of Food Sci. 47:1100. 1982
Squires, E., "Simultaneous Analysis of Multiple Sperm Attributes by Flow Cytometry , Diagnostic Techniques and Assisted Reproductive Technology, The Veterinary Clinics of North America, Equine Practice, Vol. 12, No. 1, April 1996, pp127 - 130.
Squires, E.L, Moran, D.M., Farlin, ME., Jasko, D.J., Keefe, T.J., Meyers, S.A., Figueiredo, E., McCue, P.M. and Jochle, W. 1994. Effect of dose of GnRH analogue on ovulation in mares. Theriogenology. 41:757-769.
Squires, E.L., "Early Embryonic Loss in Equine Diagnostic Ultrasonography", 1 st Ed. pp 157-163 Eds Rantanen &

McKinnon. Williams and Wilkins, Baltimore, Maryland (1998)
Squires, E.L., et al, "Cooled and frozen stallion semen", Bulletin No. 9, Colorado State University, Ft. Collins, CO. (1999)
Stellflug, J. N., D. K. Ran, R. D. Randel, and Eo L. Moody. "Plasma estrogens in peri-parturient cow." Therio 10:269. 1978
Stevenson, J. S., M. W. Smith, J. R. Jaeger, L. R. Corah, and D. G. Lefever. "Detection of estrus by visual observation and radiotelemetry in peripubertal, estrus-synchronized beefheifers." J. Anim. Sci. 74:729. 1996
Story, C. E., R. J. Rasby, R. T. Clark, and C. T. Milton. "Age of calf at weaning of spring-calving beef cows and the effect on cow and calf performance and production economics." J. Anim. Sci. 78:1403. 2000
Sullivan, J.J., Parker, W.G. and Larson, LL. 1973. Duration of estrus and ovulation time in nonlactating mares given human chorionic gonadotropin during three successive estrous periods. J.A.V.M.A. 162:895-898.
Swanson, E. W. "Future research on problems of increasing meat production by early calving." Comm. Eur. Commun., Eur. 5545.1975. The Early Calving of Heifers and its Impact on Beef Production.
Taljaard, T.L., Terblanche, S.J., Bertschinger, H.J. and Van Vuuren, L.J. 1991. The effect of the laparoscopic insemination technique on the oestrus cycle of the ewe. J. S Afr. Vet. Assoc. 62(2):60-61.
Tatum, J. D., G. C. Smith, B. W. Berry, C. E. Murphey, F. L. Williams, and Z. L. Carpenter. "Carcass characteristics, time on feed and cooked beef palatability attributes." J. Anim. Sci. 50:833. 1980
Taylor, C.S., Moore, A.J. Thiessen, R.B. and Bailey, C.M., AFRC Animal Breeding Research Organisation, West Mains Road, Edinburg EH9 3JQ, "Efficiency of Food Utilization in Traditional and Sex-Controlled Systems of Beef Production", pp 401-440.
Taylor, S. C. S., A. J. Moore, R. B. Thiessen, and C. M. Bailey. "Efficiency of food utilization in traditional and sex-controlled systems of beef-production." Animal Production 40:401. 1985
Tervit, H.R., et al., "Successful Culture In Vitro of Sheep and Cattle Ova", Agricultural Research Council, Unit of Reproduction Physiology and Biochemistry, University of Cambridge, 1972, p. 493-497.
Unruh, J. A. "Effects of endogenous and exogenous growth-promoting compounds on carcass composition, meat quality and meat nutritional-value." J. Anim. Sci. 62:1441. 1986
US Application , 09/454,488, entitled "Improved Flow Cytometer Nozzle and Flow Cytometer Sample Handling Methods", filed December 3, 1999.
US Application , 60/238,294, entitled "Hysteroscopic Insemination of Mares" filed October 5, 2000.
US Application , 09/448,643, entitled "Multiple Sexed Embryo Production System for Mammals", filed November 24, 1999.
US Application , 09/511,959 entitled "Methods For Improving Sheath Fluids and Collection Systems For Sex-Specific Cytometer Sorting of Sperm", filed February 23, 2001.
US Application 09/001,394, entitled "Sheath Fluids and Collection Systems for Sex-Specific Cytometer Sorting of

Sperm", filed on December 31, 1997, 87 total pages which includes four drawings.
US Application 09/015, 454, entitled "System for Improving Yield of Sexed Embryos in Mammals", filed on January 29, 1998, 59 total pages which includes drawings.
US Application 60/211093, entitled "Integrated System for Herd Management Using Sexed Semen", filed June 12, 2000.
US Application entitled "System For Separating Frozen-Thawed Sperm Cells Into X-Chromosome And Y-Chromosome Bearing Populations", filed November 28, 2000.
US Application Serial Number 60/094,720, entitled "System for Low Dose Insemination of Equines", filed July 30, 1998.
US Application Serial Number 60/113,143, entitled "Equine Insemination System", December 18, 1998.
US Application Serial Number 60/203,089, entitled "Detector System for Resolving Small Differences in Photo-generated Signal", filed May 9, 2000.
US Application Serial Number 60/211093, entitled "Integrated System for Herd Management Using Sexed Semen", filed June 12, 2000.
US Application Serial Number 60/224,050., entitled "Integrated System for Herd Management With Terminal-Cross Program Using Sexed Semen", filed August 9, 2000.
USDA "Official United States standards for grades of carcass beef." Agric, Marketing Serv., USDA .Washington, DC. 1997
Vazquez, J. et al., "Nonsurgical Uterotubal Insemination in the Mare", Proceedings of the 44th Annual Convention of the American Association of Equine Practitioners, Baltimore, Maryland, December 6-9, 1998, Vol. 44, pp 68-69
Vazquez, J., et al., "A.I. in Porcos; New Strategy for Deep Insemination with Low Number of Spermatozoa Using a Non-surgical Methodology", 14 th International Congress on Animal Reproduction, Vol. 2, Stockhlom, July, 2000, p. 289.
Vazquez, J., et al., "Development of a Non-surgical Deep Intra Uterine Insemination Technique", IV International Conference on Boar Semen Preservation, Maryland, August, 1999, p 35 and photo of display board.
Vazquez, J., et al., "Successful Low-Dose Insemination by a Fiberoptic Endoscope Technique in the Sow ", Proceedings Annual Conference of the International Embryo Transfer Society, Netherlands, Theriogenology, Vol. 53, January, 2000, pp. 201.
Vazquez, J., et al., "Hypoosmotic Swelling Test as Predictor of the Membrane Integrity in Boar Spermatozo", Boar Semen Preservation IV, IVth International Conference on Boar Semen Preservation, Maryland, pp. 263.
Vidament, M., Dupere, A.M., Julienne, P., Evain, A., Noue, P. and Palmer, E. 1997. Equine frozen semen freezeability and fertility field results. Theriogenology. 48:907.
Vincent, B. C., S. D. M. Jones, L. E. Jeremiah, M. A. Price, and J. A. Newman. "Carcass characteristics and meat quality of once-calved heifers." Canadian J. Anim. Sci. 71:311. 1991
Voss, J.L. and Pickett, B.W. 1976. Reproductive management of the broodmare. C.S.U. Exp. Sta. Anim. Reprod. Lab. Gen. Series. Bull. 1-12
Voss, J.L., Pickett, B.W., Burwash, L.D. and Daniels, W.H. 1974. Effect of human chorionic gonadotropin on

duration of estrous cycle and fertility of normally cycling, nonlactating mares. J.A.V.M.A. 165:704-706.
Voss, J.L., Squires, E.L., Pickett, B.W., Shideler, R.K. and Eikenberry, D.J. 1982. Effect of number and frequency of inseminations on fertility in mares. J. Reprod. Fertil. Suppl. 32:53-57.
Waggoner, A. W., M. E. Dikeman, I. R. Brethour, and K. E. Kemp. "Performance, carcass, cartilage calcium, sensory and collagen traits of longissimus muscles of open versus 30-month-old heifers that produced one calf." I. Anim. Sci. 68:2380. 1990
Welch G.R., et al., 1994. Fluidic and optical modifications to a FACS IV for flow sorting of X- and Y-chromosome bearing sperm based on DNA. Cytometry 17 (suppl. 7): 74.
Welch, G., et al., "Flow Cytometric Sperm Sorting and PCR to Confirm Separation of X- and Y- Chromosome Bearing Bovine Sperm , Animal Biotechnology, 6 (2), 131-139, 1995, pp 131 - 139.
Wheeler, T. L., L. v. Cundiff, and R. M. Koch. "Effect of marbling degree on beef palatability in Bos- Taurus and Bos-Indicus cattle." J. Anim. Sci. 72:3145. 1994
Wickersham, E. W. and L. H. Schultz. "Influence of age at first breeding on growth, reproduction, and production of well-fed holstein heifers." J. Dairy Sci. 46:544. 1963
Wilson, C.G., Downie, C.R., Hughes, J.P. and Roser, J.F. 1990. Effects of repeated hCG injections on reproductive efficiency in mares. Eq. Vet. Sci. 4:301-308.
Wilson, M.S. 1993. Non-surgical intrauterine artificial insemination in bitches using frozen semen. J.Reprod. Fert Suppl. 47:307-311.
Woods, J. and Ginther, O.J. 1983. Recent studies related to the collection of multiple embryos in mares. Theriogenology. 19:101 - 108.
Woods, J., Bergfelt, D.R. and Ginther, O.J. 1990. Effects of time of insemination relative to ovulation on pregnancy rate and embryonic-loss rate in mares. Eq. Vet. J. 22(6):410-415.
XP-002103478, File Biosis, one page.

Además, para cada término utilizado debe entenderse que a no ser que su utilización en esta solicitud sea inconsistente con tal interpretación, la definiciones comunes del diccionario deben ser entendidas tal como son incorporadas para cada término de todas sus definiciones, términos alternativos y sinónimos tales como los contenidos en el diccionario Random House Webster's Unabridged, la segunda edición que es incorporada a la presente a modo de referencia. Sin embargo a cada uno de lo mencionado anteriormente, para la extensión de tal información o

divulgación incorporada a modo de referencia debe ser considerada inconsistente con el patentamiento de estas invenciones cuyas divulgaciones son expresamente no consideradas realizadas por el solicitante.

Además, a no ser que el contexto lo requiera de otra manera, debe entenderse que el término comprende variaciones tales como comprendiendo o comprenden, están dirigidas para significar la inclusión de un elemento mencionado o paso o grupo de elementos o pasos pero no la exclusión de cualquier otro elemento o paso o grupo de elementos o pasos. Tales términos deben ser interpretados en su forma más expansiva para alcanzar para otorgar al solicitante la cobertura legal mas amplia posible en países tales como Australia y lo similar.

De esta manera el solicitante debe entender que tiene el soporte para reivindicar al menos: i) cada uno de los procedimientos de pigmentación, separación, aislamiento, inseminación o fertilización tal como es descrito aquí y divulgado, ii) los métodos relacionados divulgados y descritos, iii) variaciones similares y equivalentes o incluso implícitos de cada uno de estos dispositivos y métodos, iv) aquellos diseños alternativos los cuales realizan cada uno de las funciones ilustradas que son divulgadas y descritas, v) aquellos diseños alternativos y métodos los cuales realizan cada una de las funciones ilustradas las cuales son implícitas para realizar aquella la cual es divulgada y descrita, vi) cada característica, componente y fase ilustrada como invención separada e independiente, vii) las solicitudes mejoradas por varias disposiciones o componentes divulgados, viii) los productos resultantes producidos por tales disposiciones y componentes, ix) métodos y aparatos especialmente descritos aquí

anteriormente y con referencia a cualquiera de los ejemplos acompañantes, y x) varias combinaciones y permutaciones de cada uno de los elementos divulgados.

Las reivindicaciones mencionadas en esta solicitud son aquí incorporadas a modo de referencia como parte de la descripción de la invención, y el solicitante se reserva expresamente el derecho de utilizar todos o una porción de tal contenido incorporado en tales reivindicaciones como descripción adicional para soportar cualquiera de todas las reivindicaciones o cualquier elemento o componente de las mismas, y el solicitante además se reserva expresamente el derecho de mover cualquier porción de, o la totalidad de, el contenido incorporado de tales reivindicaciones o cualquier elemento componente de las mismas, desde la descripción hasta las reivindicaciones o viceversa en medida que sea necesario para definir el objeto por el cual se pretende protección por medio de esta solicitud o por cualquier solicitud subsiguiente de continuación, división o solicitud de mejor con respecto a la misma, o para obtener cualquier beneficio, reducción de tasas, en base a o para cumplir con las leyes, reglas o regulaciones de patentes de cualquier país o tratado, y dicho contenido incorporado a modo de referencia permanecerá vigente durante la totalidad de la vigencia de esta solicitud incluyendo cualquier solicitud subsiguiente de continuación, división, o solicitud adicional o de mejora referida a la misma o cualquier extensión o re-concesión de la misma.

REIVINDICACIONES

Habiendo así especialmente descripto y determinado la naturaleza de la presente invención y la forma como la misma ha de ser llevada a la práctica, se declara reivindicar como de propiedad y derecho exclusivo:

1. Un método para transportar oocitos que comprende los pasos de:

- a. recolectar oocitos a partir del mamífero hembra;
- b. ingresar los oocitos en un medio de fertilización
- c. transferir los oocitos en dicho medio de fertilización dentro de una cámara;
- d. sellar dicha cámara que contiene dichos oocitos;
- e. transferir al menos una cánula dentro de un elemento de incubación;
- f. establecer condiciones de incubación dentro de dicho elemento de incubación;
- g. sellar dicho elemento de incubación;
- h. transportar dichos oocitos a partir de dicho mamífero hembra dentro de dicha cánula dentro de dicho elemento de incubación.

*cauon es
al
element
de incub.*

2. Un método para transportar oocitos tal como se describe en la reivindicación 1, en donde dicho mamífero hembra seleccionado de un grupo consistente de primates o humanos, bovinos, ovinos, equinos, porcinos y delfines.

3. Un método para transportar oocitos tal como se describe en la reivindicación 1, en donde dicho medio de fertilización comprende tiroides modificados de medio suplementado con 0.6% de suero de albúmina bovino, 20 µg de

da

heparina por mililitro de medio de tiroides, y una concentración de 5 mili-molar de cafeína.

4. Un método para transportar oocitos tal como se describe en la reivindicación 1, en donde entre alrededor de 10 y alrededor de 15 de dichos oocitos son contenidos dentro de alrededor de 50 micro-litros de medio de fertilización. JCI

5. Un método para transportar oocitos tal como se describe en la reivindicación 1, en donde dicha cánula tiene elementos de abertura sellados por calor. Ja.

6. Un método para transportar oocitos tal como se describe en la reivindicación 5, en donde dicha cánula tiene un volumen interior de alrededor de 0.25 mililitros.

7. Un método para transportar oocitos tal como se describe en la reivindicación 1, en donde dicho elemento de incubación presenta elementos de abertura sellables.

8. Un método para transportar oocitos tal como se describe en la reivindicación 7, en donde dicho elemento de incubación presenta un tubo de vidrio.

9. Un método para transportar oocitos tal como se describe en la reivindicación 1, en donde dichas condiciones de incubación comprenden una atmósfera de 5% de dióxido de carbono en aire y una temperatura de 39 °C dentro de dicho elemento de incubación.

10. Un método para transportar oocitos tal como se describe en la reivindicación 1, que comprende además el pase

de transferir células de esperma a dicha cánula que contiene dichos oocitos en dicho medio de fertilización.

11. Un método para transportar oocitos tal como se describe en la reivindicación 10, en donde dicho paso de transferir células de esperma a dicha cánula que contiene dichos oocitos comprende establecer una concentración de células de esperma en dicho medio de fertilización de alrededor de 1 millón a alrededor 2 millones por mililitro de medio de fertilización.

12. Un método para transportar oocitos, tal como se describe en la reivindicación 1, que comprende además el paso de separar espermatozoides dentro de poblaciones enriquecidas que contienen cromosomas X y cromosomas Y.

caparte .
después de
10 etapas

13. Un método para transportar oocitos tal como se describe en la reivindicación 12, que comprende además el paso de transferir células de esperma separadas en dicha cámara que contiene oocitos.

14. Un método para transportar oocitos tal como se describe en la reivindicación 13, en donde dicho paso de transferir células de esperma separadas hacia dicha cánula que contiene oocitos comprende establecer una concentración de dichas células de esperma separadas en dicho medio de fertilización de alrededor de 1 millón a 2 millones por mililitro de medio de fertilización.

15. Un método para transportar oocitos tal como se describe en la reivindicaciones 10, 11, 12, 13 y 14, que comprende además el paso de transferir dicha cánula que

= 12

contiene dichos oocitos en dicha concentración de dichas células de esperma hacia dicho elemento de incubación.

16. Un método para transportar oocitos tal como se describe en la reivindicación 15, que comprende además el paso de establecer condiciones de fertilización dentro de dicho elemento de incubación. *cuales??*

17. Un método para transportar oocitos de acuerdo con lo que se describe en la reivindicación 16, en donde dicho paso de establecer condiciones de fertilización dentro de dicho elemento de incubación comprende una atmósfera de 5% de dióxido de carbono en aire a una temperatura de alrededor 37 °C y alrededor de 41 °C durante un tiempo de 18 horas a alrededor de 20 horas.

18. Un método para transportar oocitos tal como se describe en la reivindicación 16, que comprende además el paso de transportar dichos oocitos en dichas condiciones de fertilización.

19. Un método para transportar oocitos tal como se describe en la reivindicación 18, que comprende además el paso de fertilizar al menos alguno de dichos oocitos durante el transporte.

20. Un método para fertilizar oocitos, que comprende los pasos de:

a. recolectar células de esperma a partir de un mamífero macho;

b. transferir dichas células de esperma a una cánula que contiene una pluralidad de oocitos en un medio de

es el método de transporte (cubierta)

fertilización, en donde dicho oocitos son de un mamífero hembra de la misma especie que dicho mamífero macho;

c. sellar dicha cánula que contiene dichas células de esperma y dichos oocitos;

d. transferir dicha cánula que contiene dichas células de esperma y dichos oocitos hacia dentro del elemento de incubación.

e. establecer unas condiciones de incubación dentro de dicho elemento de incubación;

f. sellar dicho elemento de incubación;

g. fertilizar al menos un oocito en dicha cánula.

21. Un método para fertilizar oocitos de acuerdo con la reivindicación 20, en donde dicho mamífero hembra seleccionado de un grupo que consiste de primates, humanos, bovinos, ovinos, equinos, porcinos y delfines.

22. Un método para transportar oocitos tal como se describe en la reivindicación 20, en donde dicho medio de fertilización comprende un medio de tiroides modificado suplementado con 0.6% de suero de albúmina de bovino, 20 μ g de heparina por mililitro de medio de tiroides, y una concentración de 5 mili-molar de cafeína.

= 9¹³

23. Un método para transportar oocitos tal como se describe en la reivindicación 20, en donde entre alrededor de 10 a 15 de dichos oocitos son contenidos dentro de alrededor de 50 micro-litros de dicho medio de fertilización.

= 4

24. Un método para transportar oocitos tal como se describe en la reivindicación 20, en donde dicha cánula tiene/ elementos de abertura sellables por calor.

= 5

25. Un método para transportar oocitos tal como se describe en la reivindicación 24, en donde cánula tiene un volumen desde alrededor de 0.25 mililitros.

26. Un método para transportar oocitos tal como se describe en la reivindicación 20, en donde dicho elemento de incubación tiene elementos de abertura sellables.

27. Un método para transportar oocitos tal como se describe en la reivindicación 26, en donde dicho elemento de incubación comprende un tubo de vidrio.

28. Un método para transportar oocitos tal como se describe en la reivindicación 20, en donde dichas condiciones de incubación comprenden una atmósfera del 5% de dióxido de carbono en aire y una temperatura de 39°C dentro de dicho elemento de incubación.

29. Un método para transportar oocitos tal como se describe en la reivindicación 20, que además comprende el paso de transferir dichas células de esperma hacia dicha cánula que contiene dichos oocitos en dicho medio de fertilización.

30. Un método para transportar oocitos tal como se describe en la reivindicación 29, en donde dicho paso de transferir células de esperma a dicha cánula que contiene dichos oocitos comprende establecer una concentración de células de esperma en dicho medio de fertilización de alrededor de 1 millón a 2 millones por mililitro de medio de fertilización.

31. Un método para transportar oocitos tal como se describe en la reivindicación 21, que comprende además el paso de separar dichas células de esperma dentro de poblaciones enriquecidas que contienen cromosoma X y cromosoma Y.

32. Un método para transportar oocitos tal como se describe en la reivindicación 21, que comprende además el paso de transferir células de esperma separadas hacia dicha que contiene oocitos.

33. Un método para transportar oocitos tal como se describe en la reivindicación 32, en donde dicho paso de transferir células de esperma separadas hacia dicha cánula que contiene oocitos comprende establecer una concentración de dichas células de esperma separadas en dicho medio de fertilización de alrededor de 1 millón a alrededor de 2 millones por mililitro de medio de fertilización.

34. Un método para transportar oocitos tal como se describe en la reivindicación 29, 30, 31, 32 o 33, que comprende además el paso de transferir dicha cánula que contiene dichos oocitos en dicha concentración de dichas células de esperma hacia dicho elemento de incubación.

35. Un método para transportar oocitos tal como se describe en la reivindicación 34, que comprende además el paso de establecer condiciones de fertilización dentro de dicho elemento de incubación.

36. Un método para transportar oocitos tal como se describe en la reivindicación 35, en donde dicho paso de

establecer condiciones de fertilización dentro de dicho elemento de incubación comprende una atmósfera del 5% de carbono de óxido en aire a una temperatura de alrededor de 37 °C y alrededor de 41 °C por un tiempo de alrededor de 18 horas a alrededor de 20 horas.

37. Un método para transportar oocitos tal como se describe en la reivindicación 35, que comprende además el paso de transportar dichos oocitos en dichas condiciones de fertilización.

38. Un método para transportar oocitos tal como se describe en la reivindicación 37, que comprende además el paso de fertilizar al menos algunos de dicho oocitos durante el transporte.

39. Un método para transportar oocitos tal como se describe en la reivindicación 37, que comprende además el paso de remover los oocitos fertilizados desde dicha cánula.

40. Un método para transportar oocitos tal como se describe en la reivindicación 37, que comprende además el paso de implantar dichos oocitos fertilizados dentro de un mamífero hembra.

41. Un método para transportar oocitos tal como se describe en la reivindicación 37, en donde dicho paso implantar dichos oocitos fertilizados dentro de un mamífero hembra comprende implantar dichos oocitos fertilizados dentro del mamífero hembra de la misma especie que dicho mamífero macho.

42. Un método para transportar oocitos tal como se describe en la reivindicación 37, en donde dicho paso de implantar dichos oocitos fertilizados dentro del mamífero hembra comprende implantar oocitos fertilizados dentro de diferentes especies de dicho mamífero hembra que de dicho mamífero macho.

43. Un mamífero que presenta un sexo predeterminado de acuerdo con las reivindicaciones 35, 36, 37, 38, 39, 40, 41 o 42.

44. Una disposición de incubación portátil comprende:

a. una cánula configurada para contener un medio de fertilización dentro del cual una pluralidad de oocitos a partir de un mamífero hembra y células de esperma a partir de un mamífero macho son transferidos.

b. un elemento incubador configurado para encapsular dicha cánula que contiene un medio de fertilización dentro de dichos oocitos a partir de dicha hembra y dichas células de esperma desde dicho mamífero macho que han sido transferidas;

c. un medio ambiente de incubación mantenido dentro de dicho elemento incubador que comprende:

i. una atmósfera de incubación de alrededor de 5% de dióxido de carbono en aire;

ii. una temperatura entre alrededor de 37 °C y alrededor de 41 °C.

45. Una disposición de incubación portátil tal como se describe en la reivindicación 44, en donde dicho mamífero seleccionado del grupo consiste primates, humanos, bovinos, ovinos, equinos, marsopas y delfines.

46. Una disposición de incubación portátil tal como se describe en la reivindicación 44, en donde dicho medio de fertilización comprende un medio de tiroides suplementado con 0.6 % de suero bovino de albúmina, 20 miligramos de heparina por mililitro de medio de fertilización, y una concentración de 5 mili-molar de cafeína.

47. Una disposición de incubación portátil tal como se describe en la reivindicación 44, en donde dicha pluralidad de oocitos a partir del mamífero hembra comprende alrededor de 10 oocitos a alrededor de 15 oocitos por 50 micro-litros de medios de fertilización.

48. Una disposición de incubación portátil tal como se describe en la reivindicación 44, donde dichas células de esperma de dicho mamífero macho tienen una concentración de alrededor de un millón a alrededor de dos millones por ml. de dicho medio de fertilización.

49. Una disposición de incubación portátil tal como se describe en la reivindicación 44, donde dichas células de esperma comprenden células de esperma separadas en base a si tienen cromosomas x o si tienen cromosomas y.

50. Una disposición de incubación portátil tal como se describe en la reivindicación 44, donde dicho elemento de incubación comprende un tubo de vidrio.

51. Una disposición de incubación portátil tal como se describe en la reivindicación 44, donde dicho entorno de incubación es mantenido entre alrededor de 18 horas y alrededor de 20 horas.

52. Una disposición de incubación portátil tal como se describe en la reivindicación 44, que comprende además un elemento de transporte para mover dicha disposición de incubación portátil la distancia entre las dos ubicaciones.

53. Una disposición de incubación portátil tal como se describe en la reivindicación 52, donde la fertilización de dichos oocitos de una hembra con dichas células de esperma de dicho mamífero macho dentro de dicho medio de fertilización contenido en dicha cánula ocurre durante el transporte de dicha disposición de incubación portátil.

54. Una disposición de incubación portátil tal como se describe en la reivindicación 53, donde dichas dos ubicaciones comprenden una instalación de separación de células de esperma que tiene una primera ubicación y un mamífero hembra dentro del cual los oocitos fertilizados son implantados y que tiene una segunda ubicación.

55. Una disposición de incubación portátil tal como se describe en la reivindicación 44, donde dicha cánula que está configurada para contener un medio de fertilización dentro del cual se transfiere una pluralidad de oocitos desde un mamífero hembra y células de esperma de un mamífero macho, tiene un volumen de 0,25 ml.

56. Un método para fertilización in vitro de oocitos, que comprende las etapas de:

- a. recolectar oocitos de un mamífero hembra;
- b. disponer una pluralidad de oocitos en un medio de fertilización que contiene tanto aminoácidos esenciales como aminoácidos no esenciales;

c. inseminar dichos oocitos con células de esperma recolectadas de un mamífero macho de la misma especie que dicho mamífero hembra;

d. establecer los oocitos inseminados en condiciones de incubación dentro de un elemento de incubación; y

e. fertilizar por lo menos uno de dichos oocitos.

57. Un método para fertilización in vitro de oocitos tal como se describe en la reivindicación 56 donde dicho medio de fertilización está seleccionado del grupo que consiste de un medio químicamente definido, SOF, o TALP suplementado con un medio Eagles.

58. Un método para fertilización in vitro de oocitos tal como se describe en la reivindicación 56 donde dicho mamífero hembra está seleccionado del grupo que consiste de primates, humanos, bóvidos, ovinos, equinos, porcinos y delfines.

59. Un método para fertilización in vitro de oocitos tal como se describe en la reivindicación 56 donde dichos aminoácidos no esenciales tienen una concentración en dicho medio de fertilización que está además suplementada con aminoácidos esenciales.

60. Un método para fertilización in vitro de oocitos tal como se describe en la reivindicación 56, que incluye una pluralidad de oocitos en un medio de fertilización que contiene tanto aminoácidos esenciales como no esenciales y que comprende disponer alrededor de diez oocitos a alrededor de quince oocitos en alrededor de 50 mL de medio de fertilización.

61. Un método para fertilización in vitro de oocitos tal como se describe en la reivindicación 56 donde dicha etapa de establecer oocitos en condiciones de incubación dentro de dicho elemento de incubación comprende una atmósfera de dióxido de carbono en aire al 5% a una temperatura de entre alrededor de 37 °C y alrededor de 41 °C durante alrededor de 18 horas a alrededor de 20 horas.

conino

62. Un método para fertilización in vitro de oocitos tal como se describe en la reivindicación 56 donde dicha etapa de inseminación de dichos oocitos con células de esperma recolectadas de un mamífero macho de la misma especie que dicho mamífero hembra comprende establecer una concentración de dichas células de esperma separadas en dicho medio de fertilización de alrededor de un millón a alrededor de dos millones por mL de medio de fertilización.

63. Un método para fertilización in vitro de oocitos tal como se describe en la reivindicación 56 donde dichas células de esperma son separadas en base a que contienen cromosomas x o que contienen cromosomas y.

como?

RESUMEN

Una disposición de fertilización in-vitro para utilizar exitosamente espermatozoides separados en poblaciones que contienen cromosomas X y poblaciones que contienen cromosomas Y para inseminación. La disposición de fertilización in-vitro incluye un medio de fertilización que puede acortar el tiempo desde la inseminación hasta la segmentación y un incubador portátil para el transporte de oocitos en maduración y oocitos inseminados que comprende una cánula 19 y un elemento de incubación 20 que puede ser sellado con una tapa 22.



FORMATO DE DIGITALIZACIÓN
RELACION DE ANEXOS



Royal
Technologies S.A.
Desarrollo de Software y Servicios

122

Expediente No		03054825	No de Folio
Item		No de unidades	
1	CD		✓
2	DVD		
3	REVISTA		
4	LIBRO		
5	PERIODICO		
6	DISQUETES		
7	SOBRE DE MANILA	✓	✓
8	SOBRE BLANCO TAMAÑO CARTA	✓	
9	SOBRE BLANCO TAMAÑO OFICIO		
10	OTROS:		

Observaciones:

Art Final

SUPERINDUSTRIA Y COMERCIO
Radicacion : 02054425 00000000 Folios: 123.
Fecha (AMD): 2003-06-27 17:50:59
NIT : 800176089 Trámite : 011 PCT-PATENT 379 FASE INCI 411 PRESENTAC
Dependencia: 8020 DIVISION DE NUEVAS CREACIONES

RECIBO OFICIAL DE CAJA : 03 - 44,020
FECHA : JUNIO 24 DE 2003

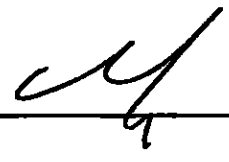
CONSIGNACION

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
CAVELIER ABOGADOS	CONSIGNACION	BANCO POPULAR	050-00110-6	11076229	24/06/2003	26,973,000.00

CONCEPTO

CANT. CONTABILISTICO	CONCEPTO	TOTAL CONCEPTO
1 50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	400,000.00
	TOTAL :	\$ 400,000.00

SON: CUATROCIENTOS MIL PESOS

RESPONSABLE : _____ 

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

Qdo 13534-841-003-0

REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL TRAMITE

		USUARIO	RADICADOR
PATENTE DE INVENCION	☒ MODELO DE UTILIDAD	()	()
- Indicación de que se solicita la concesión de una patente.		()	()
- Datos de identificación del solicitante o de la persona que se presenta la solicitud.		()	()
- Descripción de la invención.		()	()
- Dibujos de ser estos pertinentes.		()	()
- Comprobante de Pago de las tasas establecidas.			
COMPLETA	()	INCOMPLETA	()
DISEÑO INDUSTRIAL ()			
- Indicación de que se solicita el registro de Diseño Industrial		()	()
- Datos de identificación del solicitante o de la persona que presenta la solicitud.		()	()
- Representación gráfica y fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño.		()	()
- Comprobante de pago de las tasas establecidas		()	()
COMPLETA	()	INCOMPLETA	()
ESQUEMA DE TRAZADO ()			
- Indicación de que solicita el registro de un Esquema de trazado.		()	()
- Datos de identificación del solicitante o de la persona que presenta La solicitud.		()	()
- Representación gráfica del esquema de trazado		()	()
- Comprobante de pago de las tasas establecidas.		()	()
COMPLETA		INCOMPLETA	()

Firma Responsable:



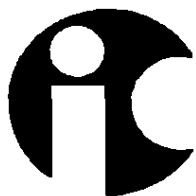
SUPERINTENDENCIA Y COMERCIO

Fecha Radicación: 03/05/2003 08:00:00

Folios: 123

Fecha (IMP): 2003-05-27 17:58:59

Trámite: 011 PCT-PATENT 379 FASE NNCI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES



2020
Bogotá DC

Doctor
EMILIO FERRERO
Apoderado
XY, INC.y Colorado State University

REFERENCIA:	Radicación No.	03-054825
	Solicitud Internacional	PCT/US2001/45237
	Fecha Inicio fase nacional	29/06/2003
	Trámite	011
	Evento	379
	Actuación	416
	Oficio No.	1217
	Folios	001

Teniendo en cuenta que la solicitud de privilegio de patente que se tramita bajo el expediente indicado en la referencia, reúne los requisitos de forma establecidos en la Decisión 486 de la Comisión de la Comunidad Andina, el Tratado de Cooperación en Materia de Patentes (PCT), el Reglamento y la Circular Unica de la Superintendencia de Industria y Comercio, se envía el extracto de la solicitud a la Oficina de Comunicaciones para efecto de su publicación en la Gaceta de Propiedad Industrial.

Cúmplase
Dado en Bogotá DC,

29 OCT. 2003


ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones

202052