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(54) Title: GLYCOFORM BIOMARKERS

(57) Abstract: The present invention relates to an assay for determining mammalian endometrial or uterine receptivity for an embryo in order to facilitate successful implantation of the embryo by natural or artificial means. The assay further discriminates between fertile and infertile mammalian females at particular secretory phases. The assay involves the detection of particular endometrial protein glycoforms using both a specific lectin and a specific antibody.



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GLYCOFORM BIOMARKERS

FILING DATA

[0001] This application is associated with and claims priority from Australian Provisional Patent Application No. 2015902891, filed on 21 July 2015, entitled "Glycoforms Biomarkers", the entire contents of which, are incorporated herein by reference.

BACKGROUND

FIELD

[0002] The present invention relates to an assay for determining mammalian endometrial or uterine receptivity for an embryo in order to facilitate successful implantation of the embryo by natural or artificial means. The assay further discriminates between fertile and infertile mammalian females at particular secretory phases.

DESCRIPTION OF THE PRIOR ART

[0003] Bibliographic details of the publication referred to by author in this specification are collected alphabetically at the end of the description.

[0004] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

[0005] Successful embryo implantation is important, not only due to the cost of the procedure, but more importantly to the psychological and emotional wellbeing of the potential mother. Whilst embryo health remains an important factor, equally or even more influential on a successful outcome is the endometrium being in a receptive state. This may be referred to as endometrial or uterine receptivity. However, developing a reliable test for such receptivity has remained elusive.

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[0006] There have been some proteomic studies in secreted fluid from the endometrium, referred to as the secretome (Casado-Vela *et al* (2009) *J. Proteome Res* 8: 4622-4632; Hannan *et al* (2009) *J. Proteome Res* 8: 1099-1103). Some of these investigations employ differential display studies of either proliferative and secretory phases (Chen *et al* (2009) *J Proteome Res* 8: 2032-2044; DeSouza *et al* (2005) *Proteomics* 5: 270-281; Dominguez *et al* (2009) *Hum. Reprod* 24: 2607-2617; Li *et al* (2011) *Fertil Steril* 95: 1161-1163; Rai *et al* (2010) *Proteomics Clin Appl* 4: 48-59) or direct comparison of fertile and infertile cohorts (Hannan *et al* (2010) *J Proteome Res* 9: 6256-6264). Whilst these studies have yielded interesting data, the main findings are multiple dysregulated forms of proteins as depicted on 2 dimensional gels as “spot trains” (for example see Packer *et al* (1998) *Electrophoresis* 19: 1872-1882; Rudd *et al* (1997) *Crit Rev Biochem Mol Biol* 32: 1-100).

[0007] Dysregulated protein forms can occur by any number of means including alterations in amino acid sequence or post translational changes. However, quantitation of a single dysregulated protein is problematic. One reason for this is that antibodies frequently are incapable of distinguishing between different post translationally modified forms of the same protein. The alternative is to use an array map or mass-spectrometric analysis which requires specialized equipment and skills, is costly and requires lengthy time frames.

[0008] There is clearly a need to develop a more efficacious assay for biomarker identification of uterine receptivity in natural and assisted reproduction.

SUMMARY

[0009] The present invention teaches the development of an assay to identify and validate markers of uterine receptivity for an embryo. Reference to “uterine receptivity” and “endometrial receptivity” are used interchangeably herein to determine the state of a uterus most receptive to successful implantation of a healthy embryo by either natural or by assisted reproduction means. The assay samples uterine lavage for particular glycoforms of one or more proteins indicative of endometrial (or uterine) receptivity.

[0010] A multiplex endometrial protein capture antibody-endometrial protein-lectin assay is taught herein to detect particular glycoforms of endometrial proteins in uterine lavage samples. Conventional sandwich ELISAs use a protein-specific primary capture antibody combined with a secondary antibody carrying a detectable label such as horseradish peroxidase or biotin. In accordance with the present invention, the secondary antibody is replaced by a lectin which provides a means to identify whether a specific glycoform is bound to the primary antibody. The lectin forms a glycoconjugate between the protein glycoform and the lectin. Hence, taught herein are particular endometrial protein-lectin binding pairs or glycoconjugates which are indicative of endometrial receptivity.

[0011] The present invention provides an assay to identify and validate glycoform biomarkers in endometrial lavage which are indicative of a receptive or non-receptive uterine environment for successful natural or assisted implantation of an embryo to or near to term. The glycoforms can also discriminate between fertile and infertile female subjects and particular secretory phase.

[0012] In an embodiment the protein-lectin pairs or glycoconjugates are identified which discriminate fertile from infertile women during mid-secretory phase of a natural cycle and those which discriminate pregnancy outcome among woman undergoing assisted reproduction.

[0013] Accordingly, one aspect of the present invention provides an assay to identify an endometrial protein glycoform biomarker in uterine lavage from a female mammal which is indicative of a level of receptivity of an endometrium for a likely successful or likely

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unsuccessful embryo implantation, the assay comprising selecting an endometrial protein having a glycoform which specifically binds to a lectin forming an endometrial protein-lectin binding pair, capturing the protein on a solid support using an immobilized primary antibody specific for the protein and then detecting the glycoform of the captured protein using the lectin specific for the glycoform wherein the lectin is labeled with a reporter molecule capable of being detected or binding a labeled antibody specific for the lectin which antibody is labeled with a reporter molecule capable of being detected. The implantation may be natural or assisted (e.g. by *in vitro* fertilization [IVF]).

[0014] Another aspect contemplates the use of a endometrial protein-lectin binding pair, wherein the lectin binds to a specific glycoform of the endometrial protein in the manufacture of an assay to detect uterine receptivity or otherwise for an embryo implant in a female mammal.

[0015] The present assay applies to human female subjects as well as non-human mammalian female animals. Hence, the assay has human clinical and veterinary applications.

[0016] Yet another aspect herein enables a method of assisted reproduction a method of assisted reproduction in a female mammal the method comprising selecting an endometrial protein having a glycoform which specifically binds to a lectin forming a protein-lectin pair, capturing the protein on a solid support using an immobilized primary antibody specific for the protein and then detecting the glycoform of the captured protein using the lectin specific for the glycoform wherein the lectin is labeled with a reporter molecule capable of being detected or binding a labeled antibody specific for the lectin which antibody is labeled with a reporter molecule capable of being detected wherein the presence or absence or level of the protein-lectin pair is indicative of whether an embryo implant should proceed.

[0017] Further enabled herein is a method of determining a female mammal that is likely to fall pregnant, the method comprising selecting an endometrial protein having a glycoform which specifically binds to a lectin forming a protein-lectin pair, capturing the protein on a solid support using an immobilized primary antibody specific for the protein

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and then detecting the glycoform of the captured protein using the lectin specific for the glycoform wherein the lectin is labeled with a reporter molecule capable of being detected or binding a labeled antibody specific for the lectin which antibody is labeled with a reporter molecule capable of being detected wherein the presence or absence or level of the protein-lectin pair is indicative of a receptive endometrial for pregnancy.

[0018] Endometrial proteins contemplated herein are defined in the subject specification and are referred to by the abbreviations ORM1, MUC5B, A2M-like (A2M-L) protein and TAFI (Table 1). The present invention extends to non-human equivalents of these proteins if they do not exist in such mammal. In addition, other potential proteins of interest are listed in Tables 19-23 of Example 9. Lectin abbreviations used herein include CONA, DBA, DSL, ECL, GSLI, GSLII, JAC, LEL, PSA, RCA, SBA, SJA, STL, ULEX, VVA and WGA (Table 2).

[0019] Particular protein-lectin binding pairs include ORM1-DBA, ORM1-CONA, A2M-L-WGA, glycodelin-RCAI, glycodelin-DBA TAFI-DBA and MUC5B-CONA.

Table 1
Endometrial protein abbreviations

Protein	Abbreviation
Orosomucoid 1	ORM 1
Mucin 5B	MUC5B
Glycodelin	Glycodelin
Alpha-2-macroglobulin-like protein	A2M-L-protein
Thrombin activatable fibrinolysis inhibitor	TAFI

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Table 2

Lectin	Abbreviation
Concavalin A	CONA
Dolichos biflorus agglutinin	DBA
Datura Stramonium lectin	DSL
Erythrina cristagalli lectin	ECL
Griffonia (Bandeiraea) simplicifolia lectin .I	GSL I
Griffonia (Bandeiraea) simplicifolia lectin II	GSL II
Jacalin	JAC
Lycopersicon esculentum lectin	LEL
Pisum sativum agglutinin	PSA
Ricinus communis agglutinin	RCA
Glycine max agglutinin.	SBA
Sophora japonica agglutinin	SJA
Solanum tuberosum lectin	STL
Ulex europaeus agglutinin I	ULEX
Vicia villosa agglutinin	VVA
Triticum vulgaris agglutinin	WGA

BRIEF DESCRIPTION OF THE FIGURES

[0020] Some Figures contain colored depictions. Copies of colored Figures can be obtained from the patentee.

[0021] **Figure 1** is a graphical representation of a histochemical evaluation of endometrial tissue performed using DBA lectin. Tissue from fertile (blue) and infertile (pink) women were collected during proliferative (Pro), early stage (ES), mid-secretory (MS) and late secretory (LS) phases. Relative staining intensity was scored in arbitrary units and the mean (n=4) intensity for each tissue type calculate as shown.

[0022] **Figure 2** is a graphical representation of pooled lavage samples from early secretory (A), infertile early secretory (B), fertile mid-secretory (C) and infertile mid-secretory (D) run on 12% w/v SDS-PAGE and stained with coomassie (Left). The samples were subject to western blot using DBA lectin (Right).

[0023] **Figure 3** is a graphical representation of lavage samples collected during the mid-secretory phase of women with proven fertility (F-MS) and those with known infertility (IF-MS) and assayed for alpha-2-macroglobulin (Luminex assay). Results for each group are presented as mean +/- standard deviation. No significant difference was found.

[0024] **Figures 4A to C** are graphical representations of uterine lavage analyzed for the concentrations of three protein glycoforms. The women were classified according to cycle stage, early secretory (es) and mid-secretory (ms), and fertility status, fertile (F) and primary infertile (pif). Results were analyzed by Kruskal-Wallis between groups of women.

[0025] **Figures 5A to D** are graphical representations showing concentrations of CONA reactive glycoforms of ORM1 and MUC5B analyzed in uterine lavage samples collected from naturally cycling fertile and primary infertile women (left side) during early (ES) and mid-secretory (MS) phases, and from IVF treated infertile women at oocyte collection human chorionic gonadotropin + 2 days (hCG+2). IVF stimulated women were grouped according to outcome of pregnancy (anta P val) or No pregnancy (ana NP val) following fresh embryo transfer in the same cycle. Results are expressed as Flurescence Intensity

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(FI). Comparison was made between groups using Mann-Whitney analysis.

[0026] **Figure 6** is a photographic representation of a protein gel image from SDS TGX stainfree gel flushing pools from proliferative fertile, proliferative infertile, secretory fertile and secretory infertile (lanes 1-4, respectively) and equal volume loading (lanes 5-8, respectively).

DETAILED DESCRIPTION

[0027] Throughout this specification, unless the context requires otherwise, the word “comprise” or variations such as “comprises” or “comprising” will be understood to imply the inclusion of a stated element or integer or method step or groups of elements or integers or method steps but not the exclusion of any other element or integer or method step or group of elements or integers or method steps.

[0028] As used in the subject specification, the singular forms “a”, “an” and “the” include plural aspects unless the context clearly dictates otherwise. Thus for example, reference to “a biomarker” or “a glycoconjugate” includes a single biomarker or glycoconjugate as well as two or more biomarkers or glycoconjugates; reference to “an embodiment” includes a single embodiment, as well as two or more embodiments; reference to “the disclosure” includes single and multiple aspects taught by the disclosure and so forth. Aspects taught and enabled herein are encompassed by the term “invention”. All such aspects are enabled within the width of the present invention. Any variants and derivatives contemplated herein are encompassed by “forms” of the invention.

[0029] The present invention is predicated in part on the identification of endometrial proteins having a particular glycosylation pattern referred to herein as a “glycoform”. The presence or absence or level or a particular endometrial protein or the ratio of levels of two or more endometrial proteins is indicative of the likelihood or otherwise of a successful embryo implantation whether by natural or assisted reproduction means. The glycoform is detected by a lectin which binds to a particular glycosylation pattern on the protein of interest to form a glycoconjugate. Hence, reference is made herein to an endometrial protein-lectin binding pair or glycoconjugate which is the lectin which binds to a particular glycoform of a selected protein. Shortened expressions such as “protein-lectin”, “protein-lectin pair” and “glycoconjugate” have the same meaning as “endometrial protein-lectin binding pair”.

[0030] Consequently, developed herein is an assay to identify endometrial protein-lectin binding pairs. Further developed is the validation of protein-lectin pairs using a modified ELISA which determines the presence, absence, level or ratio of levels of endometrial

proteins in uterine lavage at early, mid or late secretory phase (or a sub-phase in between) which enables a determination of a favorable environment in which to implant by natural or artificial means an embryo. On the assumption the embryo is healthy, if the endometrium comprises a favorable protein environment based on the glycoform of one or more proteins, then a successful embryo implantation is the likely outcome.

[0031] Hence, enable herein is an assay to identify an endometrial glycoform biomarker in uterine lavage from a female mammal which is indicative of a level of receptivity of an endometrium for a likely successful or likely unsuccessful embryo implantation, the assay comprising selecting an endometrial protein having a glycoform which specifically binds to a lectin forming an endometrial protein-lectin binding pair, capturing the protein on a solid support using an immobilized primary antibody specific for the protein and then detecting the glycoform of the captured protein using the lectin specific for the glycoform wherein the lectin is labeled with a reporter molecule capable of being detected or binding a labeled antibody specific for the lectin which antibody is labeled with a reporter molecule capable of being detected.

[0032] Further enabled herein is a method of determining a female mammal that is likely to fall pregnant, the method comprising selecting an endometrial protein having a glycoform which specifically binds to a lectin forming a protein-lectin pair, capturing the protein on a solid support using an immobilized primary antibody specific for the protein and then detecting the glycoform of the captured protein using the lectin specific for the glycoform wherein the lectin is labeled with a reporter molecule capable of being detected or binding a labeled antibody specific for the lectin which antibody is labeled with a reporter molecule capable of being detected wherein the presence or absence or level of the protein-lectin pair is indicative of a receptive endometrial for pregnancy.

[0033] Whilst the subject assay is particularly exemplified with respect to human females, the concept of the present invention equally applies to assessing the receptivity of any non-human female mammal undergoing assisted reproduction or natural cycling. Such non-human mammals include horses, cattle, sheep, pigs, deer and zoo-housed mammals including those endangered such as gorillas, monkeys, orangutans and the Tasmanian

devil. Hence, the present assay has both human clinical and veterinary applications. The assay is also useful in the monitoring of natural cycles in mammalian females such as human female subjects.

[0034] In an embodiment, however, the assay is used to assess the receptivity of a human female. Accordingly taught herein is an assay to identify an endometrial protein glycoform biomarker in uterine lavage from a human female which is indicative of a level of receptivity of an endometrium for a likely successful or likely unsuccessful embryo implantation, the assay comprising selecting an endometrial protein having a glycoform which specifically binds to a lectin forming an endometrial protein-lectin binding pair, capturing the protein on a solid support using an immobilized primary antibody specific for the protein and then detecting the glycoform of the captured protein using the lectin specific for the glycoform wherein the lectin is labeled with a reporter molecule capable of being detected or binding a labeled antibody specific for the lectin which antibody is labeled with a reporter molecule capable of being detected.

[0035] Further enabled herein is a method of determining a human female that is likely to fall pregnant, the method comprising selecting an endometrial protein having a glycoform which specifically binds to a lectin forming a protein-lectin pair, capturing the protein on a solid support using an immobilized primary antibody specific for the protein and then detecting the glycoform of the captured protein using the lectin specific for the glycoform wherein the lectin is labeled with a reporter molecule capable of being detected or binding a labeled antibody specific for the lectin which antibody is labeled with a reporter molecule capable of being detected wherein the presence or absence or level of the protein-lectin pair is indicative of a receptive endometrial for pregnancy.

[0036] Hence, the present invention provides:

- (i) an endometrial protein present or absent or at a particular level in early, mid or late secretory phase uterine lavage;
- (ii) the endometrial protein or ratios of levels of two or more endometrial proteins predicts a receptive or non-receptive uterine environment for successful embryo

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implantation by natural or artificial means;

- (iii) the endometrial protein comprises a particular glycosylation pattern referred to herein as a glycoform; and
- (iv) a lectin which specifically binds to that glycoform to form a glycoconjugate enabling detection and/or quantification of the presence, absence or level of one protein or ratio of two or more proteins enabling a predication to be made on the likelihood or otherwise of a successful embryo implantation.

[0037] As indicated above, the assay applies not only to assisted reproduction but also to the likelihood of otherwise of a natural pregnancy. Also as indicated above, a female mammal includes a human female and a non-human female mammal. A “human female” may also be referred to herein as a “woman”. Generally, the female mammal is of baby bearing age but the assay may also be useful in assessing menopausal changes and hormonal changes, especially those changes leading to a predisposition to a uterine cancer.

[0038] The sample is referred to as a “uterine lavage” or “endometrial lavage” and both terms may be used interchangeably to assess uterine receptivity for embryo implantation. The term “lavage” is not to imply any limitation as to the means of obtaining a uterine sample which includes an endometrial sample. Conveniently, a uterine lavage is the least invasive. The term “biological sample” may also be applied to the uterine/endometrial sample. The assay includes micro-arrays, macro-arrays and nano-arrays on planar or spherical solid supports. A “sample” includes but is not limited to from 0.5 µl to 50 ml.

[0039] The term “successful” means an embryo which goes to term resulting in a live birth whether natural or by caesarean section as well as the embryo developing to an age where a baby can be delivered pre-term, generally by caesarean section.

[0040] The “likeliness” or “likelihood” of a successful implantation is relative but factors such as health of the embryo, physical condition of the female subject and other factors not necessarily related to the developing fetus do affect the outcome. In an embodiment, on the assumption the embryo is healthy, the implantation procedure does not adversely effect the embryo or endometrium and the female subject remains in a healthy state, the selected

endometrial protein glycoform can successfully discriminate between a fertile female from a non-fertile female in early, mid or late secretory phase. Furthermore, pregnancy outcome can be predicted with an at least 75% success rate. By “at least 75%” means at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%. This applies to natural and assisted reproduction.

[0041] The endometrial protein can be referred to as glycoform, glycomarker, biomarker, glycoform biomarker, predictor, marker, indicator or any such term applying to a protein present in endometrial lavage. The term “glycolconjugate” applies to a protein-lectin bonded pair. The protein need not solely reside in the endometrial lavage but its presence, absence or level or its ratio of levels with at least one other protein needs to be correlated with uterine receptivity or non-receptivity for embryo implantation.

[0042] Examples of endometrial proteins, include but are not limited to orosmucoid 1 (ORM1), mucin 5B (MUC5B), glycodelin, alpha-2-macroglobulin (A2M)-like (A2M-L) protein and thrombin activatable fibrinolysis inhibitor (TAFI). Other Examples are provided in Tables 19 to 23 of Example 9. Examples of suitable lectins include concanavalin A (CONA), dolichos biflorus agglutinin (DBA), datura stramonium lectin (DSL), erythrina cristagalli lectin (ECL), griffonia (bandeiraea) simplicifolia lectin I (GSLI), griffonia (bandeiraea) simplicifolia lectin II (GSLII), jacalin (JAC), lycopersicon esculentum lectin (LEL), pisum sativum agglutinin (PSA), ricinus communis agglutinin (RCA), glucine max agglutinin (SBA), sophora japonica agglutinin (SJA), solanum tuberosum lectin (STL), ulex europaeus agglutinin I (ULEX), vicia villosa agglutinin (VVA) and triticum vulgaris agglutinin (WGA).

[0043] Examples of particular endometrial protein-lectin binding pairs include ORM1-DBA, ORM1-CONA, A2M-L-WGA, glycodelin-RCA1, glycodelin-DBA, TAFI-DBA and MUC5B-CONA.

[0044] In a particular embodiment, the endometrial protein-lectin binding pairs are A2M-L-WGA, ORM1-DBA and glycodelin-RCA1.

[0045] Accordingly, enabled herein is an assay to identify an endometrial protein

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glycoform biomarker in uterine lavage from a female mammal which is indicative of a level of receptivity of an endometrium for a likely successful or likely unsuccessful embryo implantation, the assay comprising selecting an endometrial protein from the group consisting of ORM1, MUC5B, glycodelin, A2M-like protein and TAFI from a human female or their equivalent in a non-human mammal having a glycoform which specifically binds to a lectin selected from the group consisting of CONA, DBA, DSL, ECL, GSLI, GSLII, JAC, LEL, PSA, RCA, SBA, SJA, STL, ULEX, VVA and WGA forming an endometrial protein-lectin binding pair, capturing the protein on a solid support using an immobilized primary antibody specific for the protein and then detecting the glycoform of the captured protein using the lectin specific for the glycoform wherein the lectin is labeled with a reporter molecule capable of being detected or binding a labeled antibody specific for the lectin which antibody is labeled with a reporter molecule capable of being detected.

[0046] In an embodiment the protein is selected from A2M-like protein, ORM1 and glycodelin and the corresponding lectin specific for the particular glycoform is WGA, DBA and RCA1, respectively.

[0047] Monoclonal or polyclonal antibodies may be used as the capture antibody for the endometrial protein or if needed the “tertiary” antibody. A tertiary antibody is used to bind to a lectin portion of a glycoconjugate. The use of monoclonal antibodies in an immunoassay is particularly useful because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art.

[0048] Whilst a modified ELISA is useful, there is a wide range of other immunoassay techniques available as can be seen by reference to U.S. Patent Nos. 4,016,043, 4,424,279 and 4,018,653.

[0049] The following is a description of one type of assay. An unlabeled antibody is immobilized on a solid substrate and the sample to be tested for the endometrial protein

brought into contact with the bound antibody. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-protein complex, a lectin specific to the glycoform of the protein, labeled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-protein-labeled lectin. Any unreacted material is washed away, and the presence of the protein glycoform is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of protein. This generalized technique is well known to those skilled in the art as would be any of a number of variations. In addition, a “tertiary antibody” may also be used to detect the lectin if the lectin is not labelled.

[0050] In these assays, a primary antibody having specificity for the instant protein is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, spheres, discs of microplates, or any other surface suitable for conducting an immunoassay. Microspheres are particularly useful. The binding processes are well known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the solid phase-antibody complex is washed in preparation for the test sample. An aliquot of the lavage to be tested is then added to the solid phase antibody and incubated for a period of time sufficient (e.g. 2-120 minutes or where more convenient, overnight) and under suitable conditions (e.g. for about 20°C to about 40°C) to allow binding of any subunit present in the lavage. Following the incubation period, the reaction vessel is washed and dried and incubated with a lectin specific for the glycoform of the protein. The lectin is generally linked to a reporter molecule which is used to indicate the binding of the protein to the primary antibody.

[0051] There are many variations to this assay. One particularly useful variation is a simultaneous assay where all or many of the components are admixed substantially simultaneously. Furthermore, binding of an antibody to a lectin may be employed to detect the lectin.

[0052] By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules. Examples of suitable fluorophores well known. In the case of an enzyme-based reporter molecule, an enzyme is conjugated to the lectin, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labeled lectin is added to the immobilized antibody-protein complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-protein-lectin. The substrate will react with the enzyme linked to the lectin, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of protein which was present in the sample. Again, the present disclosure extends to a substantially simultaneous assay.

[0053] Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to lectins without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labeled lectin adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic color visually detectable with a light microscope. The fluorescent labeled lectin is allowed to bind to the first antibody-protein complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the protein of interest. Immunofluorescence and enzyme immunoassay techniques are both

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very well established in the art. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

[0054] There are a range of other detection systems which may be employed including colloidal gold and all such detection systems are encompassed by the present disclosure.

[0055] The assay enabled herein may be automated or semi-automated for high throughput screening or for screening for a protein glycoforms from the one or multiple subjects. The automation is conveniently controlled by computer software.

[0056] The present disclosure further contemplates therefore web-based and non-web-based systems where data on the receptivity of a subject are provided by a client server or other architecture platform to a central processor which analyses and compares to a control and optionally considers other information such as patient age, weight and other medical conditions and then provides a report, such as, for example, a risk factor for embryo implantation failure. Hence, business method is provided whereby uterine lavage is collected in transportable tubes which is then analyzed for protein glycoforms at a defined location and the results then sent in the form of an electronic report *via* a client server or other architecture platform to a clinical care provider.

[0057] Hence, knowledge-based computer software and hardware also form part of the present disclosure. This facilitates clinical care to ascertain whether a subject has a degree of endometrial receptivity sufficient to expect a likely successful pregnancy.

[0058] In a further embodiment, the present disclosure enables kits for use with the methods described above. In one embodiment, an glycoconjugate kit is contemplated.

[0059] The immunodetection reagents of the kit may take any one of a variety of forms, including those detectable labels that are associated with or linked to the given protein glycoform, and detectable labels that are associated with or attached to lectin. Exemplary lectins are listed in Table 2.

[0060] The components of the kits may be packaged either in aqueous media or in lyophilized form.

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[0061] The container means of any of the kits generally includes at least one vial, test tube, flask, bottle, syringe or other container means, into which the testing lavage is placed, and generally, suitably aliquoted. Where a lectin is provided, the kit will also generally contain a second, third or other additional container into which this lectin may be placed. The kits taught by the present disclosure also typically include a means for immobilizes the protein of interest and any other reagents.

[0062] The present specification is also enabled for the use of an endometrial protein-lectin binding pair, wherein the lectin binds to a specific glycoform of the endometrial protein in the manufacture of an assay to detect uterine receptivity or otherwise for an embryo implant in a female mammal such as a human female.

[0063] The assay may be used in a method of assisted reproduction in a female mammal the method comprising selecting an endometrial protein having a glycoform which specifically binds to a lectin forming a protein-lectin pair, capturing the protein on a solid support using an immobilized primary antibody specific for the protein and then detecting the glycoform of the captured protein using the lectin specific for the glycoform wherein the lectin is labeled with a reporter molecule capable of being detected or binding a labeled antibody specific for said lectin which antibody is labeled with a reporter molecule capable of being detected wherein the presence or absence of the protein-lectin pair is indicative of whether an embryo implant should proceed.

[0064] In an embodiment, the female mammal is a human female. In another embodiment, the assay is used to predict receptivity for a natural pregnancy.

EXAMPLES

[0065] Aspects taught and enabled herein are further described by the following non-limiting Examples.

EXAMPLE 1

Development of lectin-modified ELSA

[0066] A primary antibody to a particular protein identified in uterine lavage is immobilized to a solid support. The solid support may be any support including a microsphere, microparticle or the side of a reaction vessel. The primary antibody is immobilized *via* its constant domains in the Fc region. A lavage sample is brought into contact with the immobilized primary antibody for a time and under conditions sufficient for a protein to which the antibody is specific binds. Unbound material and superfluent lavage fluid is washed away. A selected lectin specific for a glycoform of the captured protein is then added. The lectin is labeled with a detectable marker or label such as streptavidin, horseradish peroxidase or detection is *via* a tertiary antibody specific for the captured lectin. By using various lavage samples from fertile and infertile women, a particular glycoform of the protein which either facilitates successful implantation or is associated with embryo failure is identified.

[0067] Where microspheres are employed, the microspheres are transferred to a microcentrifuge tube and pelleted at 8000 g. The supernatant is then removed and the beads suspended in 100 mM sodium phosphate, pH 6.2, by Vortex agitation and sonication. Conjugation reagents (e.g., sulfo-NHS) and EDC are added to the microspheres and incubated with gentle mixing followed by centrifugation. Supernatant is then removed. The pelleted beads are resuspended in 50 mM MES, pH 5.0 and washed twice in the same buffer by repeated pelleting and re-suspension. The selected antibody is added to the microspheres and incubated with mixing. To optimize coupling, initially a titration of various antibody concentrations is used.

[0068] The microspheres are pelleted and PBS/Tween 20 buffer added to block further reaction sites. Antibody binding is confirmed by incubating the microspheres with serial

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dilutions of a phycoerythrin anti-species IgG detection antibody in the wells of a pre-wetted filtration plate. After suctioning off the excess liquid and washing away inbound detection antibody, the microspheres are resuspended and screened for the IgG detection antibody.

[0069] Preliminary assessment of proteins of interest is made using lavage samples from individuals with proven fertility or infertility. In each group, approximately 5-6 samples collected at early/mid secretory phase are used. The samples are each mixed with the microsphere beads conjugated with antibody to the protein of interest in a prewetted 96-well filtration plate and incubated. Replicate wells of each sample/microsphere mixture are prepared to permit secondary detection with both a biotin conjugated antibody to the protein, and with the identifying lectin also biotinylated. Following incubation of microspheres and sample, the liquid is suctioned off, and the wells washed by three applications of wash buffer, with suctioning in between. The secondary agent (antibody-biotin or lectin-biotin) is then applied and incubated. After further washing, the detection reagent, streptavidin-phycoerthythrin is added to all wells. Following incubation and exhaustive washing, the microspheres are resuspended in Bioplex sheath fluid and analysed on a Bioplex-200 instrument. The detected signal quantified as fluorescence intensity will be determined for each well. A blank well for each microsphere/secondary reagent in which sample is substituted with buffer is used to assess any background binding occurring between the secondary reagent and the immobilized capture antibody.

[0070] A graphical and t-test of results for fertile and infertile individuals is made for the total (antibody detected) and individual glycoform (lectin detected) results. Further verification studies of the most promising biomarkers are performed using larger sample cohorts.

EXAMPLE 2

Proteomic analysis of glycoconjugates

[0071] Pooled uterine lavage fluid collected from women, of known fertility or infertility, during their early-mid secretory phase is used. The two pools, fertile (F) and Infertile (IF) are utilized in parallel during all stages of analysis. Initial separation is performed by incubating each pool with a specific agarose bound lectin contained in microspin columns. The unbound protein fraction is removed following centrifugation, and used subsequently for repeated procedures on other agarose lectins. Following exhaustive washing of the spin columns with buffer, the bound glycoproteins (protein glycoforms) are eluted by incubation with a simple sugar complementary to the lectin, e.g. for DBA lectin n-acetylglucosamine is added to elute the captured glycoprotein. The isolated protein fraction is precipitated and resuspended in buffer ready for proteomic analysis.

[0072] The protein from F and IF pools captured using two different lectins is labeled using ICPL-4 reagent (Kellermann (2008) *Methods mol boil* 424: 113-123). Using ICPL-4 it is possible to label four proteomic states i.e. 4 samples for simultaneous quantitation (Lottspeich and Kellermann (2011) *Methods Mol Biol* 753: 55-64; Schmidt *et al* (2005) *Proteomic S* 5: 4-15). ICPL provides a top down approach labeling the protein prior to separation and enzymatic digestion. ICPL contains four isotopically labeled variants which respectively add a recognisable mass-shift relative to the native isotopic form. Thus, with each of four samples labeled with a unique variant, they may be combined for analysis.

[0073] Once each isolate is labeled it is separated using OFFGEL separation. Conventional 2D electrophoresis performs an initial isoelectric focussing (pI separation) with a secondary molecular weight separation of a complex protein mixture. OFFGEL has been demonstrated to provide improved proteomic coverage. Using OFFGEL the initial isoelectric separation is performed in the liquid phase. In brief, a rehydrated IPG strip is sealed within the well frame and the diluted solution (ICPL-4 labeled combination of four lectin isolates) added equally to all wells and sealed to prevent evaporation. High voltage is applied to the IPG strip and the proteins migrate until they reach a position where the pH matches their pI. After separation the proteins remain within their respective wells until

transferred for further analysis.

[0074] The contents of each well are further separated by HPLC before the proteins are digested with Glu-C and analyzed using maldi-TOF spectrometry. At this stage the ICPLquant software (Brunner *et al* (2010) *Proeomics 10*: 315-326) is used to analyze the obtained spectra to identify the proteins present but also to make a quantitative comparison of each ICPL label for each identified protein, thus providing quantitative detail as to the abundance of a glycoform. Hence, at this stage it will identify glycoforms significantly dysregulated between the paired F and IF isolates for each lectin.

EXAMPLE 3

Lectin binding to endometrial epithelium changes with cycle phase

[0075] A preliminary histochemical screen with a number of lectins on endometrial tissue taken at different phases of the menstrual cycle confirms that some lectins demonstrate a phase dependent binding to endometrial tissue (Figure 1).

EXAMPLE 4

Endometrial lectin binding changes with fertility status

[0076] A study has also demonstrated that the intensity of binding for some lectins, and hence concentration of glycoconjugates within the endometrium differs in infertile women, notably during the receptive mid-secretory phase, and importantly within the secretory sub-structure of the endometrium i.e. the luminal epithelium and glands.

EXAMPLE 5

Cyclical and fertility-related lectin binding is also seen within the endometrial secretome

[0077] To determine if the cyclic lectin binding to endometrial tissue is reflected in binding to proteins of the endometrial secretome, Western blots are performed with individual lectins on uterine lavage pooled from fertile and infertile women during the early and mid-secretory phase of their cycle. The blots of fluid from infertile women

clearly show greater amounts and differing band profiles of proteins binding DBA that have been secreted from the endometrium, indicative of an altered glycosylation profile approaching and during the critical receptive phase (Figure 2).

EXAMPLE 6

Conventional ELISA does not detect change in glycoforms

[0078] To illustrate the difficulty of utilizing identified dysregulated glycoforms as biomarkers in conventional ELISA, a comparative 2D-DIGE study of fertile and infertile women during the mid-secretory phase was further examined (Hannan *et al* (2010) *Supra*). The authors identified a number of protein spots as differentially expressed, including a number of highly abundant proteins (e.g. alpha-2-macroglobulin, antithrombin III and alpha1-antitrypsin). They performed an immunohistochemical study for alpha-2-macroglobulin; however staining intensity between fertile and infertile tissue failed to confirm their initial proteomic data, though they were able to confirm an elevated expression in secretory as opposed to proliferative tissue of fertile women. To follow through on these data, a number of endometrial lavages were collected from fertile and infertile women during the mid-secretory phase and quantified the alpha-2-macroglobulin present using a commercial Luminex assay kit (Figure 3). No significant dysregulation was apparent, a finding attributable to the assay in effect quantifying all forms of the protein and not the one glycoform of interest.

EXAMPLE 7

[0079] A selection of antibody lectin pairs was tested to determine their ability to discriminate mid-secretory lavage samples from fertile and primary idiopathic infertile women. The antibody lectin pairs tested were listed in Table 3. A number of these displayed some significance in their discrimination of fertile and infertile endometrium (Table 3).

Table 3

Data from protein Lectin pairs of interest

Protein	Lectin	P value	Significant
Orosmucoid 1	DBA	0.030	Yes*
Orosmucoid 1	ConA	0.069	Yes
Mucin 5B	ConA	0.093	Yes
Glycodelin	RCA1	0.004	Yes **
Glycodelin	DBA	0.134	No
A2M-like protein	WGA1	0.004	Yes**
TAFI	DBA	In progress	TBD

[0080] In this Example, the aim was to further maximize the performance of these biomarkers, prior to screening of a larger cohort of lavages (n = 40) [EXAMPLE 8] comprising fertile secretory v infertile early and mid-secretory phase lavages. The data are analyzed by t-test to determine significance between groups for each individual marker, together with ROC curve analyses to provide diagnostic performance data (sensitivity and specificity). In addition combinations of the markers are assessed using multivariate logistic modelling, using a leave-one out cross-validation. The resulting ROC curve analysis determine the diagnostic efficiency of combined biomarkers. Use of a leave-one out cross-validation modelling negates the need for separate ‘model’ and ‘test’ sets (and thus increased sample numbers) while still providing a robust diagnostic signature.

[0081] The most successful markers are tested on lavage samples from fertile egg donors on LH+2 to determine the impact of their hormone treatments, and on samples taken on the same day from women at the time of embryo pickup. The biomarker test results for the IVF cohorts would then be input into the signature generated previously to predict whether the endometrium is classified as receptive. These predicted results correlate with the actual result (confirmed pregnancy or otherwise) to determine the reliability of the biomarkers for determining receptivity in IVF cycles. These results are reported as a diagnostic accuracy of the biomarker tests for prediction of endometrial suitability.

Method and Rationale*Patient Cohorts*

[0082] The patient cohorts were selected to provide a detailed assessment of the ability of protein glycoform assays to determine endometrial receptivity. A larger sample set of 40 subjects comprised:

- 10 fertile mid-secretory phase (i.e. receptive)
- 10 infertile mid-secretory phase (i.e. non-receptive)
- 10 fertile early-secretory phase (i.e. non-receptive)
- 10 infertile early-secretory phase (i.e. non-receptive)

[0083] The inclusion of the early secretory phase fertile women within this sample set provides an indication whether the protein glycoform expression is mid-secretory phase receptive specific, given that during the early secretory phase it is expected that the endometrium will be non-receptive. Further the inclusion of early secretory phase infertile women would indicate if there is a dys-synchrony in their endometrial receptivity.

[0084] Additional analysis is performed on lavage samples collected from infertile women undergoing IVF treatment. The lavage is collected at hCG+2 (oocyte collection day) with patients being segregated according to cycle outcome i.e. pregnancy or no pregnancy. All women in this cohort received GnRH antagonist regimen. This sample cohort can be considered early secretory phase and thus essentially non-receptive; however consideration has been given to the knowledge that in women undergoing IVF there may be an advancement of the receptive phase of the endometrium. Additionally, it is unknown as to the exact trigger for glycosylation changes in the endometrium:

thus the IVF hormonal protocols may impact on the glycosylation profiles. The cohort comprised;

- GnRH antagonist with outcome Pregnancy
- GnRH antagonist with outcome No Pregnancy

Standardization

[0085] All assays are standardized using a pool of uterine flushing collected cross-cycle

from secondary infertile and unknown fertility women. The standard is deemed as having a concentration of 1000 arbitrary units for each assay. Standard curves are fitted to a 5 parameter fit (or 4-parameter where insufficient points were available). Antibody and lectin concentrations are listed in Table 4 below.

Assay conditions

Table 4
Assay conditions for each antibody-lectin assay

Antibody	Lectin	Lectin dilution	Sample dilution
ORM1	DBA	1:1000	1:10
Glycodelin	RCA1	1:1000	1:10
Glycodelin	DBA	1:1000	1:10
A2ML-1	WGA	1:1000	1:10
TAFI	DBA	1:1000	1:2
ORM1	Con A	1:1000	1:10
MUC5B	Con A	1:1000	1:10

Statistical Analysis

[0086] Data are analyzed by ANOVA with individual pairings further analyzed using Dunns comparison of means to indicate significance. Mann-Whitney analysis of significant group pairs is performed. Secondary ROC analysis is additionally performed.

[0087] Three assays (A2M-L-WGA, ORM1-DBA and Glycodelin-RCA1) had achieved significant results in initial testing with a small cohort of mid-secretory fertile and infertile women (Table 3). These three assays, A2M-L-WGA, ORM1-DBA and Glycodelin-RCA1, when tested with the extended cohort (n=40) again demonstrated significant difference in ANOVA analysis (p= 0.036*, 0.003** and 0.004**, respectively). Dunns comparison indicated significance between fertile and infertile women during the mid-secretory phase (Figure 4). Mann-Whitney analysis confirmed significance between mid-secretory fertile

and infertile populations for A2M-L-WGA ($p=0.0472^*$), ORM1/DBA ($p=0.0054^{**}$) and Glycodelin-RCA1 ($p=0.0007^{***}$). For all three cases the infertile mid-secretory phase samples were raised. Further, the pregulation from early to mid-secretory phase among infertile women was itself significant for ORM1/DBA and A2M-L-WGA ($p=0.0024^{**}$ and 0.0286^* respectively). In all three assays no significant differences between the fertile and infertile populations during the early secretory phase was seen, and likewise there was no altered expression among the fertile population between early and mid-secretory phases. It was noted that in the ORM1-DBA assay, with the exception of the infertile mid-secretory group, a significant proportion of samples did not give measurable values, indicative that this particular glycosylation has a high degree of specificity.

[0088] ROC analysis of the three assays performance in discriminating fertile and infertile mid-secretory women was performed using DeLong method. Results for each assay showed significant discrimination by ROC analysis.

[0089] Spearman correlation of the three glycoform markers was performed on the mid-secretory samples, with fertile and infertile analyzed separately (Table 5). For the fertile samples a strong correlation ($r=0.811$, $p=0.008$) was seen between A2M-L-WGA and Glycodelin-RCA1. However in the infertile population this correlation was not so clearly evident ($r=0.622$, $p=0.060$) however A2M-L-WGA and ORM1-DBA did correlate ($r=0.833$, $p=0.005$).

Table 5

Spearman correlation analysis was made between assays for fertile and infertile cohorts collected during the mid-secretory phase

Assay 1	Assay 2	Fertile		Infertile	
		r=	p=	r=	p=
ORM1/DBA	A2M-L/WGA	0.437	0.211	0.833	0.005
ORM1/DBA	Glycodelin/RCA1	0.396	0.267	0.644	0.052
A2M-L/WGA	Glycodelin/RCA1	0.811	0.008	0.622	0.060

[0090] In these analyses, the uterine lavage was collected from both fertile and infertile women during natural menstrual cycles. These three assays were further applied to cohorts of infertile women undergoing IVF treatment. In these women, lavage was collected at the time of oocyte harvest. This is effectively the early secretory phase being just post-ovulation. The women sampled were undergoing GnRH antagonist treatment and were classified according to their cycle outcome, i.e. pregnancy, or no pregnancy following fresh embryo transfer in the same cycle. Statistical analysis found no significant difference between these groups classified by outcome. This is unsurprising given an earlier observation that these markers showed mid-secretory and not early secretory discrimination of fertility.

[0091] Multivariate modelling of the three assays was performed using WEKA software, with the mid-secretory data input. Results with the mid-secretory fertile and infertile data produced 100% correct classification, and an AUC of 1.000. However, no test data set is available; given it is the infertile mid-secretory women who differ in expression levels of the three assays, the use of the early secretory data cannot be used as a test set of non-receptivity. Cross validation was performed in two ways; 10-fold cross validation produced 70% correct classification with AUC=0.715, and leave-one out cross validation produced 65% correct classification with AUC = 0.700. Clearly this dataset is small and a larger dataset and independent test set would provide for a more robust signature.

[0092] Analysis of a further three protein glycoforms (ORM1-CONA), MUC5B-CONA and Glycodelin-DBA) on a small cohort of mid-secretory women had indicated that while

there was some discrimination of fertile and infertile women this was not achieving a level of significance (Table 3). These protein glycoforms were further explored on the extended sample set of 40 samples, mid and early secretory, fertile and infertile women. For the two assays utilizing CONA, a significant background issue continues to make use of the assay difficult. The background arises from cross-reaction of the catcher antibodies own glycosylations with the CONA lectin. Analysis was thus performed on the fluorescence intensity as opposed to the calculated arbitrary units/mL concentration. This has the effect of reducing the level of difference as FI is not a direct linear relationship with concentration.

[0093] In the ORM1-CONA assay no significant difference was seen between fertile and infertile women during either the early or mid-secretory phase (Figure 5); however there was a significant change between cycle phases of both fertile (0.0003***) and infertile women (0.0115*). A similar pattern of discrimination was observed for the Muc5B-CONA assay with significance between cycle phases of fertile (0.0068**) and infertile (0.0089) women. Analysis of the antagonist cycle IVF patient cohort revealed a significant difference between outcomes of 'Pregnancy' and 'No pregnancy' for both the ORM1/ConA (0.0754) and Muc5B/ConA (0.0335) assays. This cycle phase data provide further evidence that glycosylation patterns do indeed alter during the receptive phase of the endometrium. Despite in the natural cycles no significant difference between fertile and infertile being evident in either early or mid-secretory phases, the data from IVF stimulation cycles does indicate a difference at hCG+2 (early secretory equivalent), adding to existing data that suggest a different response to the stimulation program by individual women.

[0094] Correlation analysis of ORM1-CONA and Muc5B-CONA showed no correlation among patient groups. Data are shown in Figure 5.

EXAMPLE 8*Identification of lection panel*

[0095] A selection of lectins with differing carbohydrate binding specificities were identified for testing and are listed in Table 6;

Table 6*Lectins panel*

LECTIN NAME	ABBREVIATION
Concavalin A	CONA
<i>Dolichos biflorus</i> agglutinin	DBA
<i>Datura Stramonium</i> lectin	DSL
<i>Erythrina cristagalli</i> lectin	ECL
<i>Griffonia (Bandeiraea) simplicifolia</i> lectin I	GSL I
<i>Griffonia (Bandeiraea) simplicifolia</i> lectin II	GSL II
Jacalin	JAC
<i>Lycopersicon esculentum</i> lectin	LEL
<i>Pisum sativum</i> agglutinin	PSA
<i>Ricinus communis</i> agglutinin	RCA
<i>Glycine max</i> agglutinin	SBA
<i>Sophora japonica</i> agglutinin	SJA
<i>Solanum tuberosum</i> lectin	STL
<i>Ulex europaeus</i> agglutinin I	ULEX
<i>Vicia villosa</i> agglutinin	VVA
<i>Triticum vulgare</i> agglutinin	WGA

Immunohistochemical Evaluation

[0096] A comparison was made of lectin binding to proliferative (non-receptive) and secretory (receptive) endometrial tissue. Each lectin listed in Table 6 was optimized according to the protocol in EXAMPLE 2, to determine a suitable concentration of lectin for examination of tissues selected from panels of fertile and infertile women (Table 7 and Table 8). In addition for each tissue a negative control in which the lectin was inhibited by pre-incubation with an appropriate blocking sugar was included. Staining of glandular and luminal epithelial cells for each tissue was scored on a scale of 0-3 assessing intensity and distribution. Scoring is based within a single independent set of slides, i.e. a score of 2 in an individual lectin is not representative of a score of 2 in a second lectin.

*Tissue panels***Table 7*****Tissue panels from fertile women for IHC studies of lectin binding***

Proliferative	Early Secretory	Mid Secretory	Late Secretory
NH739	NH886	NH778	NH800
NH521	NH675	NH727	NH226
NH458	NH381	NH333	NH141
NH240	NH181	NH158	NH125
NH477		NH507	
NH298		NH673	

Table 8***Tissue panels from infertile women for IHC studies of lectin binding***

Proliferative	Early Secretory	Mid Secretory	Late Secretory
NH957	NH586	NH864	NH837
NH932	NH379	NH658	NH544
NH811	NH318	NH510	NH301
NH730	NH132	NH260	NH291
NH220	NH655	NH438	
NH780	NH1112		
NH914			

WesternBlot Analysis

[0097] Western blot analysis of fertile and infertile proliferative and early-mid secretory phase uterine flushings was conducted with the panel of lectins (Table 9). For this purpose four pool samples were prepared (fertile proliferative, infertile proliferative, fertile early-mid secretory and infertile early-mid secretory). These pools comprised the following individual samples pooled in equal volumes. Western blotting was performed as described in Example 1.

Table 9
Individual uterine flushings pooled for western blot analysis

Pool GFI-A		Pool GFI-B		Pool GFI-C		Pool GFI-D	
Fertile Proliferative		Infertile Proliferative		Fertile early-mid secretory		Infertile early-mid secretory	
SAMPLE	AGE (yrs)	SAMPLE	AGE (yrs)	SAMPLE	AGE (yrs)	SAMPLE	AGE (yrs)
NH681	32	NH1297	30	NH635	31	NH981	31
NH765	33	NH1274	33	NH549	33	NH865	34
NH867	34	NH481	35	NH832	34	NH1311	34
NH1167	34	NH1260	35	NH448	35	NH980	35
NH763	37	NH330	37	NH115	37	NH890	37
NH283	38	NH1017	38	NH580	39	NH1332	40
NH739	40	NH224	40	NH618	40	NH658	41
NH212	41	NH643	42	NH814	41	NH862	42
MEAN	36.1	MEAN	36.3	MEAN	36.3	MEAN	36.8

[0098] Each pooled was assayed for protein concentration using BCA protein assay. Each sample was assayed in duplicate at three dilution (neat, 1:4, 1:9), and the assay repeated by two operators. Mean protein concentration was determined for each pool, and the results are shown in Table 10.

Table 10
Protein determined using BCA assay for flushings pools are shown in ug/ml. Values shown are means from duplicate assays performed at three sample dilutions, and replicated by two operators.

Flushing Pool	Mean protein concentration (ug/ml)
GFI-A	912
GFI-B	1252
GFI-C	580
GFI-D	1701

Results

[0099] Table 11 summarizes the determined optimal concentrations of lectin and inhibitor sugar used in immunohistochemistry studies. The appropriate dilution of individual lectins for immunohistochemistry was determined in initial studies. For each lectin an appropriate neutralizing sugar and its' concentration required to achieve full

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inhibition of lectin binding to tissue for use as a negative control was also determined. Table 12 summarizes the optimal concentrations for western blotting with each lectin. The appropriate dilution of individual lectins for western blotting was determined in initial studies.

Table 11
Optimal concentration of lectin and inhibitor sugar

Lectin	Lectin Dilution	Inhibitor Sugar	Sugar Concentration (µg/ml)
CONA	36000	Mannoside	50
DBA	400	Acetylgalactosamine	10
DSL	10000	Chitin hydrolysate	Neat
ECL	600	Lactose	100
GSL I	1000	Galactose	100
GSL II	300	Chitin hydrolysate	Neat
JAC	16000	Galactose	100
LEL	600	Acetylgalactosamine	10
PSA	5000	Mannoside	50
BCA	15000	Acetylgalactosamine	10
SBA	500	Acetylgalactosamine	10
SJA	400	Acetylgalactosamine	10
STL	2000	Chitin hydrolysate	Neat
ULEX	800	Fucose	100
VVA	600	Acetylgalactosamine	10
WGA	10000	Chitin hydrolysate	Neat

Table 12
Optimal concentration of lectin for Western blotting

Lectin	Dilution Factor
Con A	2000
DBA	500
DSL	1000
ECL	1000
GSL-I	1000
GSL-II	1000
JAC	1000
LEL	1000
PSA	1000
RCA	1000
SBA	500
SJA	1000
STL	1000
ULEX	1000
VVA	1000
WGA	1000

[0100] Immunohistochemistry and western blotting results are presented for each individual lectin. Photographic images are produced representative of the tissue study results. Graphical representations of staining of Luminal and Glandular epithelia are also generated. Western blots show obvious differences between fertile and infertile, proliferative and secretory pools. For each lectin two development time images are produced, the second longer exposure providing comparison of some of the lower abundance proteins, while the shorter exposure picture allows visual comparison of the highly abundant proteins.

[0101] A representative protein gel image is shown in Figure 6. The gel is loaded in similar manner as was performed for each western blot, thus the four sample pools are shown at equal protein and equal volume loading.

[0102] IHC studies show demonstrable CONA recognition throughout the cycle, however, it is seen to elevate in the mid to late secretory phase in fertile women. This elevation is less clear in the infertile cohort.

[0103] DBA staining within the luminal epithelia was sparse or zero in the proliferative phase tissues of both fertile and infertile individuals. The staining increased throughout the secretory phase and was apically positioned within the cells. The infertile women displayed greater expression, and this was indeed also true within the glandular epithelium where highly intense staining throughout the majority of glands was visible by the mid secretory phase, and visible secretions within the glands also noted. Western analysis though reactivity was weak, revealed two bands which appeared uniquely within the mid-secretory infertile pool.

[0104] Prolific staining of the apical surface of the luminal epithelia throughout the menstrual cycle. Staining also present in most glands throughout the cycle, and appearing as secretions within glands in the secretory phase tissues. No observable expression difference between fertile and infertile women. Western analysis identified a band of approximately 70kDa present in the fertile secretory pool.

[0105] Immunohistochemistry showed a consistent level of staining throughout the cycle and regardless of fertility status within the apical surface of luminal and glandular epithelium. Western analysis found only a weak signal making analysis difficult however there appeared to be no observable differences between pool samples.

[0106] Weak staining of tissue epithelium was seen, but there appeared no pattern to expression across the cycle and no evidence of altered expression between fertile and infertile cohorts. Western analysis showed very poor reactivity with GSLI.

[0107] GSLII appears to bind more strongly to secretory phase tissue from fertile women, compared with proliferative phase tissue. In addition there is an indication that infertile women showed less reactivity during the secretory phase. However, western analysis showed very poor reactivity with GSLII.

[0108] Immunohistochemistry indicates that in fertile women there is a rise in jacalin

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reactive sugars during the secretory phase compared to the proliferative. However, the infertile population appears to have a higher proliferative expression, thus obscuring any altered levels in the secretory phase. In Western analysis JAC showed good resolution . There is no clear specific staining below 50kDa. Of note is the 180kDa band which is visible only in the Fertile Proliferative pool. Additionally, a 150kDa band is visible in the Infertile secretory pool, however, it is not visible where less protein is loaded. A 50kDa band is visible in infertile pools. It is worth noting that overall staining is darker in the Fertile Proliferative pool even with equal protein loading suggestive of a greater expression of jacalin recognized sugars overall.

[0109] LEL binding reaches a maximum in the mid secretory phase of fertile women, evident in both the glandular and luminal epithelium. However there appeared no clear pattern of difference between fertile and infertile women. Western analysis identified two small molecular weight bands (21 and 22kDa) and a larger 70kDa band, prominent in all groups except the Infertile proliferative pool. Additionally, longer exposure identified a 45kDa band visible only in the Infertile proliferative pool.

[0110] Staining of glandular epithelium appears to increase in the mid secretory phase of the cycle, and there is some indication that this elevation is not so strong in the infertile cohort. There is, however, extensive stromal staining throughout the cycle. PSA Western blotting did not show differences between the pooled groups. The differences observed between groups, appear to be dependent on protein amount loaded at equal volume and are not seen with equal protein loading.

[0111] Immunohistochemical analysis found RCA-1 while strong in the proliferative phase luminal epithelium declined through to a lowest level in the mid-secretory phase of the fertile women's cycle before rising again in the late secretory. However, the infertile cohort appeared to reach its low point in the early secretory phase, and be recovering by the mid secretory. Glandular expression did not show such a clear cyclic pattern. Western blot analysis found RCA-I shows good resolution of several bands. There is no specific staining of proteins <50kDa. Some bands are apparently restricted to particular groups, however, most bands differ mainly in intensity between groups rather than being unique

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between samples. Most notably, three bands are prominent in all groups but appear very faint at 130 and 110kDa or absent at 50kDa in the Fertile Secretory pool.

[0112] Tissue analysis showed a cyclic expression pattern of SBA binding sugars notably in the glandular epithelium, where in fertile women a trough occurs at the early secretory stage, followed by an increase in the mid secretory phase. There is a suggestion that the infertile cohort may show a somewhat reduced expression throughout the cycle. In western analysis of sample pools there was no clearly visible difference in binding of the SBA lectin.

[0113] Staining of tissue with SJA was restricted to some areas of luminal epithelium and glandular epithelium however the staining was very weak. This absence of SJA binding sites was likewise reflected in western analysis where there was little signal at all.

[0114] STL showed a very uniform level of staining of tissues regardless of stage or fertility status, though actual signal strength was quite strong. In western analysis however despite a lengthy imaging of 30 minutes very little signal could be observed, perhaps indicative that the STL recognized sugars are primarily on non-secreted proteins within the tissue.

[0115] ULEX showed a very disparate binding to tissues throughout the cycle with both high and low binding samples in all stages of the cycle. ULEX blotting did not show differences between the pooled groups. The differences observed between lanes, appear to be dependent on protein loading as they are not evident in lanes where equal protein is loaded.

[0116] A small amount of luminal epithelial staining in proliferative tissue was visible but did not show any discernable increase during progression of the secretory phase. The glandular epithelia did however show a clear elevation of binding in the mid and late secretory phases of the normal fertile women. This was replicated in the infertile cohort.

[0117] There was no clear pattern to WGA binding to tissue regardless of fertility status or stage of cycle. In the western blot analysis the area of most interest is in the region around 150kDa. Analysis revealed two distinct bands in the Fertile Proliferative pool, one

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large band in the Infertile Secretory pool, while the Fertile Secretory pool showed very little staining in this region. Conversely, a 75kDa band is relatively strong in the Fertile Secretory pool in comparison with the other groups.

EXAMPLE 9

Proteomic identification of protein glycoforms

[0118] This Example relates to the proteomic identification of protein glycoforms showing unique or dysregulated expression in uterine lavage samples between fertile and infertile women in the early-midsecretory phase.

[0119] Three lectins, CONA, RCA-1 and WGA, that capture glycoproteins were selected for further investigation based on the data the previous Examples. These were applied to pooled secretory phase lavage from fertile and infertile women. Subsequently CONA analyses were repeated using individual samples. This allowed more detailed analysis of early and mid-secretory phases individually.

[0120] The initial results using pooled lavage found for each lectin, a number of dysregulated glycoproteins showing either unique or greater than 2-fold change in detected protein between fertile and infertile groups. For WGA these were 46 of 113 proteins, for RCA, 43 of 103 proteins and for CONA, 50 of 118 proteins identified.

[0121] Subsequent analysis of individual samples using CONA lectin revealed 73 of 125 protein dysregulated, 68 of 116 proteins in early secretory samples, and 64 of 96 proteins in mid secretory samples. It should be noted that the mass-spectrometry used in this analysis identifies only proteins with an appropriate N-linked carbohydrate moiety, that used for the lavage pools identifies proteins with both O and N linkages.

Proteomics Method 1

[0122] Two pools of uterine flushings were prepared, one of fertile and the other of primary infertile women. Each pool comprised samples of early secretory (n=3) and mid secretory (n=3) phase lavage, based on pathology staging of endometrial biopsies taken at

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the time of lavage collection. The pooled samples were each loaded onto a fresh spin column of lectin beads, incubated and then extensively washed to remove unbound proteins. The lectin bound proteins were then competitively eluted using an appropriate sugar solution. A second elution with acid, and final elution with SDS were performed to ensure that all proteins bound by the lectin were eluted for analysis. SDS-PAGE and western blot using the lectin of interest were performed on non-binding and elution fractions from the isolation. The sugar eluate was trypsin digested for subsequent MS analysis.

[0123] Starting and elution fractions for each isolation were analysed by SDS-PAGE and western blot with the lectin, to determine efficiency of the capture/elution steps.

[0124] This process captures intact proteins from the uterine lavage samples, and by eluting with sugars, lectin binding of both N and O linked glycosylations is reversed. In the MS analysis post trypsin digestion, it is the non-glycosylated regions of the isolated proteins which are identified.

[0125] This process was applied to three lectins; WGA, RCA-1, and CONA.

Proteomics Method 2

[0126] Individual samples of early and mid secretory staged uterine lavage samples from fertile and infertile women were trypsin digested, then mixed with the lectin of choice, and separated using a filter that isolated lectin bound glycosylated peptides from those not carrying an appropriate sugar moiety. The lectin captured peptides were released from the lectin enzymatically using PNGase cleavage and identified using MS.

[0127] This process isolates and identifies only the peptide region carrying the actual glycosylation of interest, allowing analysis of changes to specific glycosylation sites. In addition, this method can provide for future sequential lectin absorptions as only peptides, not all regions of the protein, are selectively bound at each stage.

[0128] This method was applied to CONA.

Proteomics Data Analysis

[0129] For both proteomics methods, analysis used Scaffold software to identify proteins which were either absent from one cohort *vs* the other, or showed >2-fold change in expression (based on normalized spectra count), either up or down regulated. Fisher test for Exactness was also applied and the significance value (p) reported.

Results*Wheat Germ Agglutinin*

[0130] From the 300µg protein in each of the pooled secretory flushings, 20.4µg (6.8%) was recovered by sugar elution from the fertile pool and 23.4µg (7.8%) from the infertile pool. Some protein (8.4µg) was measured in the acid elution from the fertile but not the infertile samples. Both samples were run on 1D PAGE. A 16kDa band is visible in both stain-free gels, but is absent in the lectin blots indicating it is not WGA-specific.

[0131] The results are summarized in Tables 13 to 18.

Table 13
WGA Proteomics Method 1

	Number of Proteins
Total Proteins Identified in Experiment	113
Unique 'Fertile' Proteins	15
Unique 'Infertile' Proteins	13
>2 up-regulated in infertile <i>vs</i> fertile groups	10
>2 fold down-regulated in infertile <i>vs</i> fertile groups	8

Ricinus Agglutinin

[0132] From the 290µg of protein in the fertile secretory flushings, 25.2µg (8.7%) was recovered in the sugar elution. From the 553µg of protein in the infertile secretory flushings, 42.6µg (7.7%) was recovered in the sugar elution. A small amount of protein (3µg) was measured in the acid elution of the fertile flushing isolation. Following analysis 1D PAGE, this is visible as a 28kDa band in the stain-free gel, but is absent in the lectin

blot indicating it is not RCA-specific.

Table 14

RCA1 Proteomics Method 1

	Number of Proteins
Total Proteins Identified in Experiment	103
Unique 'Fertile' Proteins	15
Unique 'Infertile' Proteins	19
>2 up-regulated in infertile vs fertile groups	14
>2 fold down-regulated in infertile vs fertile groups	5

Concavalin A

[0133] From the 290µg of protein in the fertile secretory flushings, 19.8µg (6.8%) was recovered in the sugar elution. From the 553µg of protein in the infertile secretory flushings, 46.8µg (8.5%) was recovered in the sugar elution. ID PAGE analyses were conducted. Unbound proteins are seen in the stain-free gel images in both groups. There is a small amount of CONA-specific 50kDa protein visible in the fertile pool lectin blot and in the infertile pool lectin blot. All isolated CONA-specific glycoproteins were eluted in the sugar solution in both the fertile and the infertile isolations.

[0134] Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any to or more of said steps of features.

Table 15
CONA Proteomics Method 1

	Number of Proteins
Total Proteins Identified in Experiment	118
Unique 'Fertile' Proteins	24
Unique 'Infertile' Proteins	16
>2 up-regulated in infertile vs fertile groups	13
>2 fold down-regulated in infertile vs fertile groups	7

Table 16
CONA Proteomics Method 2 (individual samples analyzed)

	Number of Proteins
Total Proteins Identified in Experiment	125
Unique 'Fertile' Proteins	14
Unique 'Infertile' Proteins	39
>2 up-regulated in infertile vs fertile groups	22
>2 fold down-regulated in infertile vs fertile groups	8

Secretory Fertile (n=6) v Secretory Infertile (n=6)

Table 17
Early Secretory Fertile (n=3) v Early Secretory Infertile(n=3)

	Number of Proteins
Total Proteins Identified in Experiment	116
Unique 'Fertile' Proteins	12
Unique 'Infertile' Proteins	15
>2 up-regulated in infertile vs fertile groups	12
>2 fold down-regulated in infertile vs fertile groups	29

Table 18***Mid Secretory Fertile (n=3) v Mid Secretory Infertile(n=3)***

	Number of Proteins
Total Proteins Identified in Experiment	96
Unique 'Fertile' Proteins	10
Unique 'Infertile' Proteins	27
>2 up-regulated in infertile vs fertile groups	12
>2 fold down-regulated in infertile vs fertile groups	15

Protein lists are shown in Tables 19 to 23. Identifications are color coded as shown:

Unique identifications in Fertile Samples
 >2-fold down-regulated in Infertile Samples
 >2-fold up-regulated in Infertile Samples
 Unique identifications in Infertile Samples

Table 19
WGA Protein Analysis Results (Proteomics method 1)

Protein	Accession Number	Molecular Weight	Fold	PK	Fertile	P-value
sp O75882-2 ATRIN isoform 2 of Attractin Tax_id=9606 [Homo sapiens]	O75882-2 (+1)	141 kDa	2	1	2	0.48
sp P05164 MPO Myeloperoxidase Tax_id=9606 [Homo sapiens]	P05164 (+2)	84 kDa	2	10	20	0.038
sp O08380 LGALS3BP Galectin-3 binding protein Tax_id=9606 [Homo sapiens]	O08380 (+1)	65 kDa	2	2	4	0.32
tr B4DUV1 cDNA FL53207, highly similar to Homo sapiens fibulin 1 (FBLN1), transcript variant C, mRNA Tax_id=9606 [Homo sapiens]	B4DUV1	70 kDa	2.8	4	11	0.05
sp P24158 PRIN3 Myeloblastin Tax_id=9606 [Homo sapiens]	P24158	28 kDa	3	1	3	0.3
tr Q8NE11 Putative uncharacterized protein Tax_id=9606 [Homo sapiens]	Q8NE11	25 kDa	3	1	3	0.3
sp P02749 APOH Beta-2-glycoprotein 1 Tax_id=9606 [Homo sapiens]	P02749	38 kDa	4	2	8	0.047
sp P51884 LUM Lumican Tax_id=9606 [Homo sapiens]	P51884	38 kDa	5	2	10	0.016
sp O00592 PODXL Podocalyxin Tax_id=9606 [Homo sapiens]	O00592 (+5)	59 kDa	INF	0	1	0.49
sp P00915 CA1 Carbonic anhydrase 1 Tax_id=9606 [Homo sapiens]	P00915 (+6)	29 kDa	INF	0	3	0.12
sp P01880 IGHD Ig delta chain C region Tax_id=9606 [Homo sapiens]	P01880 (+2)	42 kDa	INF	0	5	0.028
sp P02774 GC Vitamin D-binding protein Tax_id=9606 [Homo sapiens]	P02774 (+5)	53 kDa	INF	0	1	0.49
sp P05154 SERPINA5 Plasma serine protease inhibitor Tax_id=9606 [Homo sapiens]	P05154	46 kDa	INF	0	2	0.24
sp P06731 CEACAM5 Carcinoembryonic antigen-related cell adhesion molecule 5 Tax_id=9606 [Homo sapiens]	P06731 (+2)	77 kDa	INF	0	3	0.12

sp P08123 COL1A2 Collagen alpha-2(I) chain Tax_id=9606 [Homo sapiens]	P08123	129 kDa	INF	0	8	0.0032
sp P08758 ANXA5 Annexin A5 Tax_id=9606 [Homo sapiens]	P08758 (+3)	36 kDa	INF	0	4	0.057
sp P10599 TXN Thioredoxin Tax_id=9606 [Homo sapiens]	P10599 (+1)	12 kDa	INF	0	3	0.12
sp P13611 VCAN Versican core protein Tax_id=9606 [Homo sapiens]	P13611 (+3)	373 kDa	INF	0	2	0.24
sp P31947 SFN 14-3-3 protein sigma Tax_id=9606 [Homo sapiens]	P31947 (+2)	28 kDa	INF	0	3	0.12
sp P54108 CRISP3 Cysteine-rich secretory protein 3 Tax_id=9606 [Homo sapiens]	P54108 (+3)	28 kDa	INF	0	3	0.12
sp P60709 ACTB Actin, cytoplasmic 1 Tax_id=9606 [Homo sapiens]	P60709 (+6)	42 kDa	INF	0	4	0.057
sp Q16610 ECM1 Extracellular matrix protein 1 Tax_id=9606 [Homo sapiens]	Q16610 (+1)	61 kDa	INF	0	5	0.028
sp Q9UBC9 SPRR3 Small proline-rich protein 3 Tax_id=9606 [Homo sapiens]	Q9UBC9 (+2)	18 kDa	INF	0	3	0.12
sp P00739 HPR Haptoglobin-related protein Tax_id=9606 [Homo sapiens]	P00739 (+1)	39 kDa	0.2	5	1	0.12
sp P03952 KLKB1 Plasma kallikrein Tax_id=9606 [Homo sapiens]	P03952 (+3)	71 kDa	0.3	3	1	0.33
sp P08185 SERPINA6 Corticosteroid-binding globulin Tax_id=9606 [Homo sapiens]	P08185 (+1)	45 kDa	0.3	6	2	0.16
sp P10909 CLU Clusterin Tax_id=9606 [Homo sapiens]	P10909 (+3)	52 kDa	0.5	17	9	0.1
tr B7ZLE5 FN1 FN1 protein Tax_id=9606 [Homo sapiens]	B7ZLE5 (+1)	247 kDa	0.5	17	9	0.1
sp P02679 FGG Fibrinogen gamma chain Tax_id=9606 [Homo sapiens]	P02679 (+1)	52 kDa	0.5	20	10	0.062
sp P04114 APOB Apolipoprotein B-100 Tax_id=9606 [Homo sapiens]	P04114 (+1)	516 kDa	0.5	99	46	<0.00010
sp P05543 SERPINA7 Thyroxine-binding globulin Tax_id=9606 [Homo sapiens]	P05543	46 kDa	0.5	2	1	0.52

[Homo sapiens] tr E4DPQ0 cDNA FLJ54471, highly similar to Complement C1r subcomponent [EC 3.4.21.41] Tax_id=9606 [Homo sapiens] tr Q6GMW4 IGL@ protein Tax_id=9606 [Homo sapiens]	B4DPQ0 Q6GMW4	82 kDa 25 kDa	0.5 0.5	2 4	1 2	0.52 0.36
p A8K2U0 A2ML1 Alpha-2-macroglobulin-like protein 1 Tax_id=9606 [Homo sapiens]	A8K2U0 (+1)	161 kDa	0	1	0	0.51
sp O75636 FCN3 Ficolin-3 Tax_id=9606 [Homo sapiens]	O75636 (+2)	33 kDa	0	3	0	0.13
sp P00734 F2 Prothrombin Tax_id=9606 [Homo sapiens]	P00734	70 kDa	0	2	0	0.26
sp P00748 F12 Coagulation factor XII Tax_id=9606 [Homo sapiens]	P00748 (+1)	68 kDa	0	1	0	0.51
sp P01861 IGHG4 Ig gamma-4 chain C region Tax_id=9606 [Homo sapiens]	P01861	36 kDa	0	4	0	0.068
sp P02649 APOE Apolipoprotein E Tax_id=9606 [Homo sapiens]	P02649	36 kDa	0	3	0	0.13
sp P02743 APCS Serum amyloid P-component Tax_id=9606 [Homo sapiens]	P02743	25 kDa	0	2	0	0.26
sp P04430 Ig kappa chain V-J region BAN Tax_id=9606 [Homo sapiens]	P04430	12 kDa	0	2	0	0.26
sp P06396 GSN Gelsolin Tax_id=9606 [Homo sapiens]	P06396	86 kDa	0	1	0	0.51
sp P09871 C1s Complement C1s subcomponent Tax_id=9606 [Homo sapiens]	P09871 (+1)	77 kDa	0	9	0	0.0023
sp P22792 CPN2 Carboxypeptidase N subunit 2 Tax_id=9606 [Homo sapiens]	P22792	61 kDa	0	3	0	0.13
sp P69891 HBG1 Hemoglobin subunit gamma-1 Tax_id=9606 [Homo sapiens]	P69891 (+5)	16 kDa	0	3	0	0.13
tr I1VZV6 HBA1 Hemoglobin alpha 1 Tax_id=9606 [Homo sapiens]	I1VZV6	15 kDa	0	1	0	0.51

RCA-1 Protein Analysis Results (Proteomics method 1)

Protein	Accession Number	Molecular Weight	Fold Change	Pf	Fertile	P-value
sp P01617 Ig kappa chain V-II region TEW Tax_id=9606 [Homo sapiens]	P01617 (+1)	12 kDa	INF	0	2	0.19
sp P02760 AMBP Protein AMBP Tax_id=9606 [Homo sapiens]	P02760	39 kDa	INF	0	4	0.036
sp P02765 AHSG Alpha-2-HS-glycoprotein Tax_id=9606 [Homo sapiens]	P02765 (+2)	39 kDa	INF	0	1	0.44
sp P06731 CEACAM5 Carcinoembryonic antigen- related cell adhesion molecule 5 Tax_id=9606 [Homo sapiens]	P06731 (+2)	77 kDa	INF	0	3	0.082
sp P13611 VCAN Versican core protein Tax_id=9606 [Homo sapiens]	P13611 (+3)	373 kDa	INF	0	10	0.00024
sp P15941 MUC1 Mucin 1 Tax_id=9606 [Homo sapiens]	P15941 (+24)	122 kDa	INF	0	2	0.19
sp P20061 TCN1 Transcobalamin-1 Tax_id=9606 [Homo sapiens]	P20061	48 kDa	INF	0	1	0.44
sp P24158 PRTN3 Myeloblastin Tax_id=9606 [Homo sapiens]	P24158	28 kDa	INF	0	4	0.036
sp P60709 ACTB Actin, cytoplasmic 1 Tax_id=9606 [Homo sapiens]	P60709 (+6)	42 kDa	INF	0	1	0.44
sp P80188 LCN2 Neutrophil gelatinase-associated lipocalin Tax_id=9606 [Homo sapiens]	P80188 (+3)	23 kDa	INF	0	1	0.44
sp Q14508 WFDC2 WAP four-disulfide core domain protein 2 Tax_id=9606 [Homo sapiens]	Q14508	13 kDa	INF	0	3	0.082
sp Q16610 ECM1 Extracellular matrix protein 1 Tax_id=9606 [Homo sapiens]	Q16610 (+1)	61 kDa	INF	0	1	0.44
tr B2R4F9 cDNA, FLJ92074, highly similar to Homo sapiens progesterone-associated endometrial protein (placental protein 14, pregnancy-associated endometrial alpha-2 globulin, alpha uterine protein)	B2R4F9	19 kDa	INF	0	7	0.0029

(PAEP), mRNA Tax_id=9606 [Homo sapiens] tr B4DUV1 cDNA FLJ53207, highly similar to Homo sapiens fibulin 1 (FBLN1), transcript variant C, mRNA Tax_id=9606 [Homo sapiens]	B4DUV1	70 kDa	INF	0	12	<0.00010
tr B4E1B2 cDNA FLJ53691, highly similar to Serotransferrin Tax_id=9606 [Homo sapiens]	B4E1B2	75 kDa	INF	0	3	0.082
tr Q8TCD0 putative uncharacterized protein Tax_id=9606 [Homo sapiens]	Q8TCD0	26 kDa		1	2	0.4
sp P25311 AZGP1 Zinc-alpha-2-glycoprotein Tax_id=9606 [Homo sapiens]	P25311	34 kDa	2.3	3	7	0.086
sp P05164 MPO Myeloperoxidase Tax_id=9606 [Homo sapiens]	P05164 (+2)	84 kDa	2.7	11	30	0.0001
sp P05543 SERPINA7 Thyroxine binding globulin Tax_id=9606 [Homo sapiens]	P05543	46 kDa	3	2	6	0.075
sp P01024 C3 Complement C3 Tax_id=9606 [Homo sapiens]	P01024	187 kDa	7	2	14	0.00036
tr B7ZLE5 FN1 FN1 protein Tax_id=9606 [Homo sapiens]	B7ZLE5 (+1)	247 kDa	0.06	36	2	<0.00010
sp P03952 KLKB1 Plasma kallikrein Tax_id=9606 [Homo sapiens]	P03952 (+3)	71 kDa	0.2	6	1	0.12
sp P04004 VTN vitronectin Tax_id=9606 [Homo sapiens]	P04004	54 kDa	0.2	9	2	0.078
sp P04114 APCB Apolipoprotein B-100 Tax_id=9606 [Homo sapiens]	P04114 (+1)	516 kDa	0.2	122	26	<0.00010
sp P08185 SERPINA6 Corticosteroid-binding globulin Tax_id=9606 [Homo sapiens]	P08185 (+1)	45 kDa	0.2	10	2	0.052
sp P02679 FGG Fibrinogen gamma chain Tax_id=9606 [Homo sapiens]	P02679 (+1)	52 kDa	0.3	22	6	0.012
sp P05090 APOD Apolipoprotein D Tax_id=9606 [Homo sapiens]	P05090 (+1)	21 kDa	0.3	3	1	0.41
sp Q08380 LGALS3BP Galectin-3-binding protein Tax_id=9606 [Homo sapiens]	Q08380 (+1)	65 kDa	0.3	9	3	0.16
sp P02671 FGA Fibrinogen alpha chain	P02671 (+2)	95 kDa	0.4	11	4	0.14

[illegible]

[Homo sapiens] sp P06702 S100A9 Protein S100-A9 Tax_id=9606 [Homo sapiens]	P06702 (+1)	0	4	0	0.1
sp P08246 ELANE Neutrophil elastase Tax_id=9606 [Homo sapiens]	P08246	0	2	0	0.32
sp P09871 C15 Complement C15 subcomponent Tax_id=9606 [Homo sapiens]	P09871 (+1)	0	2	0	0.01
sp P15169 CPN1 Carboxypeptidase N catalytic chain Tax_id=9606 [Homo sapiens]	P15169	0	2	0	0.32
sp P19823 ITIH2 Inter-alpha-trypsin inhibitor heavy chain H2 Tax_id=9606 [Homo sapiens]	P19823 (+3)	0	7	0	0.018
sp P22792 CPN2 Carboxypeptidase N subunit 2 Tax_id=9606 [Homo sapiens]	P22792	0	3	0	0.18
sp Q14624 ITIH4 Inter-alpha-trypsin inhibitor heavy chain H4 Tax_id=9606 [Homo sapiens]	Q14624 (+5)	0	2	0	0.32
tr A8K008 cDNA FLJ78387 Tax_id=9606 [Homo sapiens]	A8K008	0	1	0	0.56
tr B4DPQ0 cDNA FLJ54471, highly similar to Complement C1r subcomponent (EC 3.4.21.41) Tax_id=9606 [Homo sapiens]	B4DPQ0	0	10	0	0.0032
tr Q6GMX6 IGH@ IGH@ protein Tax_id=9606 [Homo sapiens]	Q6GMX6	0	1	0	0.56

Table 20
CONA Protein Analysis Results (Proteomics method 1)

Protein	Accession Number	Molecular Weight	Fold Change	PiF	Fertiliz.	P values
ens ENSBTAP000000022763 ENSBTAG000000017121 transcript:ENSBTAT000000022763 gene_biotype:protein_coding transcript_biotype:protein_coding Tax_id=9913 [Bos taurus]	ENSBTAP000000022763	59 kDa	INF	0	5	0.018
sp P02774 GC_Vitamin_D-binding_protein Tax_id=9606 [Homo sapiens]	P02774 (+5)	53 kDa	INF	0	1	0.45
sp P03973 SLPI_Antileukoprotease Tax_id=9606 [Homo sapiens]	P03973	14 kDa	INF	0	3	0.089
sp P04083 ANXA1_Annexin_A1 Tax_id=9606 [Homo sapiens]	P04083 (+2)	39 kDa	INF	0	7	0.0035
sp P04272 ANXA2_Annexin_A2 Tax_id=9913 [Bos taurus]	P04272 (+8)	39 kDa	INF	0	3	0.089
sp P04792 HSPB1_Heat shock protein beta-1 Tax_id=9606 [Homo sapiens]	P04792 (+2)	23 kDa	INF	0	1	0.45
sp P05109 S100A8_Protein S100-A8 Tax_id=9606 [Homo sapiens]	P05109	11 kDa	INF	0	2	0.2
sp P06702 S100A9_Protein S100-A9 Tax_id=9606 [Homo sapiens]	P06702 (+1)	13 kDa	INF	0	9	0.0007
sp P06731 CEACAM5_Carcinoembryonic antigen- related cell adhesion molecule 5 Tax_id=9606 [Homo sapiens]	P06731 (+2)	77 kDa	INF	0	3	0.089
sp P06733 ENO1_Alpha-enolase Tax_id=9606 [Homo sapiens]	P06733 (+2)	47 kDa	INF	0	3	0.089
sp P08123 COL1A2_Collagen alpha-2(I) chain Tax_id=9606 [Homo sapiens]	P08123	129 kDa	INF	0	7	0.0025
sp P08758 ANXA5_Annexin A5 Tax_id=9606 [Homo sapiens]	P08758 (+3)	36 kDa	INF	0	4	0.04

sapiens}						
sp P10599 TXN Thioredoxin Tax_id=9606 [Homo sapiens]	P10599 (+1)	12 kDa	INF	0	1	0.45
sp P13611 VCAN Versican core protein Tax_id=9606 [Homo sapiens]	P13611 (+3)	373 kDa	INF	0	4	0.04
sp P15924 DSP Desmoplakin Tax_id=9606 [Homo sapiens]	P15924 (+4)	332 kDa	INF	0	3	0.089
sp P20160 AZU1 Azurocidin Tax_id=9606 [Homo sapiens]	P20160	27 kDa	INF	0	2	0.2
sp P29508 SERPINB3 Serpin B3 Tax_id=9606 [Homo sapiens]	P29508 (+1)	45 kDa	INF	0	7	0.0035
sp P31947 SFN 14-3-3 protein sigma Tax_id=9606 [Homo sapiens]	P31947 (+2)	28 kDa	INF	0	2	0.2
sp P60709 ACTB Actin, cytoplasmic 1 Tax_id=9606 [Homo sapiens]	P60709 (+6)	42 kDa	INF	0	8	0.0016
sp P62935 PP1A Peptidyl-prohyl cis-trans isomerase A Tax_id=9913 [Bos taurus]	P62935 (+7)	18 kDa	INF	0	2	0.2
sp P68103 EFF1A1 Elongation factor 1-alpha 1 Tax_id=9913 [Bos taurus]	P68103 (+13)	50 kDa	INF	0	2	0.2
sp Q01469 FABP5 Fatty acid-binding protein, epidermal Tax_id=9606 [Homo sapiens]	Q01469	15 kDa	INF	0	2	0.2
sp Q08380 LGALS3BP Galectin-3-binding protein Tax_id=9606 [Homo sapiens]	Q08380 (+1)	65 kDa	INF	0	1	0.45
tr B4DUV1 cDNA FLJ53207, highly similar to Homo sapiens fibulin 1 (FBIN1), transcript variant C, mRNA Tax_id=9606 [Homo sapiens]	B4DUV1	70 kDa	INF	0	19	<0.000010
tr B2R4F9 cDNA, FLJ92074, highly similar to Homo sapiens progesteragen-associated endometrial protein (placentalprotein 14, pregnancy-associated endometrial alpha-2-globulin, alpha uterine protein) {PAEP}, mRNA Tax_id=9606 [Homo sapiens]	B2R4F9	19 kDa	2	4	8	0.11
tr Q6GMW4 IGL@ IGL @ protein Tax_id=9606 [Homo sapiens]	Q6GMW4	25 kDa	2	2	4	0.25

sp P05164 MPO Myeloperoxidase Tax_id=9606 [Homo sapiens]	P05164 (+2)	84 kDa	2.1	11	23	0.0056
sp P02760 AMBP Protein AMBP Tax_id=9606 [Homo sapiens]	P02760	39 kDa	2.3	3	7	0.028
sp A8K2U0 A2ML1 Alpha 2 macroglobulin-like protein 1 Tax_id=9606 [Homo sapiens]	A8K2U0 (+1)	161 kDa	2.5	2	5	0.15
tr Q5JP19 DKF7p686D0114 Putative uncharacterized protein DKF7p686D0114 Tax_id=9606 [Homo sapiens]	Q5JP19 (+2)	30 kDa	4	1	4	0.13
sp P80188 LCN2 Neutrophil gelatinase-associated lipocalin Tax_id=9606 [Homo sapiens]	P80188 (+3)	23 kDa	7	1	7	0.017
sp P02679 FGG Fibrinogen gamma chain Tax_id=9606 [Homo sapiens]	P02679 (+1)	52 kDa	0.2	25	4	0.00041
sp P04114 APOB Apolipoprotein B-100 Tax_id=9606 [Homo sapiens]	P04114 (+1)	516 kDa	0.2	83	17	<0.00010
sp P00734 F2 Prothrombin Tax_id=9606 [Homo sapiens]	P00734	70 kDa	0.3	11	3	0.066
sp P19823 ITIH2 Inter-alpha-trypsin inhibitor heavy chain H2 Tax_id=9606 [Homo sapiens]	P19823 (+3)	106 kDa	0.3	29	8	0.003
tr B7ZLE5 FN1 FN1 protein Tax_id=9606 [Homo sapiens]	B7ZLE5 (+1)	247 kDa	0.3	33	10	0.0029
sp P02675 FGB Fibrinogen beta chain Tax_id=9606 [Homo sapiens]	P02675	56 kDa	0.4	22	9	0.055
sp P04003 C4BPA C4b-binding protein alpha chain Tax_id=9606 [Homo sapiens]	P04003 (+1)	67 kDa	0.4	25	9	0.022
sp P19827 ITIH1 Inter-alpha-trypsin inhibitor heavy chain H1 Tax_id=9606 [Homo sapiens]	P19827 (+1)	101 kDa	0.4	25	9	0.022
sp Q14624 ITIH4 Inter-alpha-trypsin inhibitor heavy chain H4 Tax_id=9606 [Homo sapiens]	Q14624 (+5)	103 kDa	0.4	20	8	0.061
tr B4E1Z4 CFB Complement factor B Tax_id=9606 [Homo sapiens]	B4E1Z4	141 kDa	0.4	24	9	0.03
sp P05543 SERPINA7 Thyroxine-binding globulin Tax_id=9606 [Homo sapiens]	P05543	46 kDa	0.5	4	2	0.45

sp P13671 C6 Complement component C6 Tax_id=9606 [Homo sapiens]	P13671 (+1)	105 kDa	0.5	6	3	0.37
tr Q8TCD0 Putative uncharacterized protein Tax_id=9606 [Homo sapiens]	Q8TCD0	26 kDa	0.5	2	1	0.58
sp O75636 FCN3 Ficolin-3 Tax_id=9606 [Homo sapiens]	O75636 (+2)	33 kDa	0	2	0	0.31
sp O75882-2 ATR1 isoform 2 of Attractin Tax_id=9606 [Homo sapiens]	O75882 2 (+1)	141 kDa	0	2	0	0.31
sp P00739 HPR Haptoglobin-related protein Tax_id=9606 [Homo sapiens]	P00739 (+1)	39 kDa	0	5	0	0.052
sp P00748 F12 Coagulation factor XII Tax_id=9606 [Homo sapiens]	P00748 (+1)	68 kDa	0	2	0	0.31
sp P01031 C5 Complement C5 Tax_id=9606 [Homo sapiens]	P01031	188 kDa	0	6	0	0.029
sp P01233 CGB Chorogonadotropin subunit beta Tax_id=9606 [Homo sapiens]	P01233 (+10)	18 kDa	0	4	0	0.093
sp P02649 APOF Apolipoprotein F Tax_id=9606 [Homo sapiens]	P02649	36 kDa	0	6	0	0.029
sp P02746 C1QB Complement C1q subcomponent subunit B Tax_id=9606 [Homo sapiens]	P02746 (+2)	27 kDa	0	1	0	0.55
sp P04430 Ig kappa chain V-1 region BAN Tax_id=9606 [Homo sapiens]	P04430	12 kDa	0	4	0	0.093
sp P05090 APOD Apolipoprotein D Tax_id=9606 [Homo sapiens]	P05090 (+1)	21 kDa	0	3	0	0.17
sp P08185 SERPINA5 Corticosteroid-binding globulin Tax_id=9606 [Homo sapiens]	P08185 (+1)	45 kDa	0	6	0	0.029
sp P09871 C1S Complement C1s subcomponent Tax_id=9606 [Homo sapiens]	P09871 (+1)	77 kDa	0	8	0	0.0087
sp P10643 C7 Complement component C7 Tax_id=9606 [Homo sapiens]	P10643 (+3)	94 kDa	0	4	0	0.093
sp Q06033 ITIH3 Inter-alpha-trypsin inhibitor heavy chain H3 Tax_id=9606 [Homo sapiens]	Q06033 (+1)	100 kDa	0	2	0	0.31
sp Q96PD5 PGLYRP2 N-acetylmuramoyl-L-alanine Tax_id=9606 [Homo sapiens]	Q96PD5 (+2)	62 kDa	0	2	0	0.031

amidase Tax_id=9606 [Homo sapiens]					
tr B4DPQ0 cDNA FLJ54471, highly similar to					
Complement C1r subcomponent (EC 3.4.21.41)	B4DPQ0	82 kDa	0	13	0
Tax_id=9606 [Homo sapiens]					0.00044

Table 21

Concavalin A Proteomics Secretory fertile v Secretory Infertile

Identified Proteins {125}	Accession Number	Molecular Weight	Fold Change	P value
Alpha-1-acid glycoprotein 1 Tax_id=9606 [Homo sapiens]	ORM1 (+1)	23 kDa	0	0.44
sulfate proteoglycan 2 (Versican) variant (Fragment) Tax_id=9606 [Homo sapiens]	Chondroitin (+9)	372 kDa	0	0.0013
transcript:ENST00000416334				
gene_biotype:protein_coding				
transcript_biotype:protein_coding Tax_id=9606 [Homo sapiens]	ens ENSP00000414674 ENSG00000135318 (+8)	26 kDa	0	0.083
Isoform Somatic-2 of Angiotensin-converting enzyme Tax_id=9606 [Homo sapiens]	sp P12821-2 ACE (+2)	130 kDa	0	0.036
Complement component C8 alpha chain Tax_id=9606 [Homo sapiens]	C8A	65 kDa	0	0.44
Alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase 1 Tax_id=9606 [Homo sapiens]	sp Q9NSC7 ST6GALNAC1 (+2)	68 kDa	0	0.44
transcript:ENST00000548934				
gene_biotype:protein_coding				
transcript_biotype:protein_coding Tax_id=9606 [Homo sapiens]	ens ENSP00000446799 ENSG00000135678 (+7)	78 kDa	0	0.19
Isoform Short of Beta-glucuronidase Tax_id=9606 [Homo sapiens]	sp P08236-2 GUSB (+2)	69 kDa	0	0.44
BPI fold-containing family B member 1 Tax_id=9606	BPIFB1 (+1)	52 kDa	0	0.44

[Homo sapiens] Isoform 3 of CD109 antigen Tax_id=9606 [Homo sapiens] transcript:ENST00000567027 gene_biotype:protein_coding transcript_biotype:nonsense_mediated_decay Tax_id=9606 [Homo sapiens] HP protein Tax_id=9606 [Homo sapiens] transcript:ENST00000546391 gene_biotype:protein_coding transcript_biotype:protein_coding Tax_id=9606 [Homo sapiens] Transcubalamin-1 Tax_id=9606 [Homo sapiens] Tax_id=9606 [Homo sapiens] transcript:ENST00000380947 gene_biotype:protein_coding transcript_biotype:nonsense_mediated_decay Tax_id=9606 [Homo sapiens] Zinc-alpha-2-glycoprotein Tax_id=9606 [Homo sapiens] Golgi membrane protein 1 Tax_id=9606 [Homo sapiens] Isoform H14 of Myeloperoxidase Tax_id=9606 [Homo sapiens] Azurocidin Tax_id=9606 [Homo sapiens] protein (Fragment) Tax_id=9606 [Homo sapiens] Putative uncharacterized protein DKFZp686D23123 Tax_id=9606 [Homo sapiens] Alpha-1-acid glycoprotein 2 Tax_id=9606 [Homo sapiens] Beta-2-glycoprotein 1 Tax_id=9606 [Homo sapiens] Antithrombin-4II Tax_id=9606 [Homo sapiens]	sp Q6VHK3-3 CD109 (+2) ens ENSP00000457521 ENSG00000213614 (+7) tr Q6PE18 HP ens ENSP00000446530 ENSG00000011465 (+6) TCN1 Glycodefin (+1) ens ENSP00000370334 ENSG00000181143 (+5) sp P25311 AZGP1 (+1) sp Q8N814 GOLM1 (+3) sp P05164-2 MPO (+2) sp P20160 AZU1 tr H7C469 Uncharacterized (+5) DKFZp686D23123 (+9) ORM2 APOH (+1) sp P01008 SERPINC1	73 kDa 36 kDa 25 kDa 19 kDa 47 kDa 16 kDa 133 kDa 34 kDa 45 kDa 73 kDa 27 kDa 40 kDa 61 kDa 23 kDa 38 kDa 52 kDa	0 0 0 0 0 0.1 0.2 0.3 0.3 0.5 0.5 0.5 0.5 2 2 2	0.19 0.44 0.0001 0.44 0.44 0.0034 0.06 0.22 0.22 0.093 0.23 0.41 0.41 0.47 0.34 0.59
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Cathepsin L2 Tax_id=9606 [Homo sapiens]	CTSL2 (+1)	37 kDa	2	0.39
Serum paraoxonase/arylesterase 1 Tax_id=9606 [Homo sapiens]	sp P27169 PON1 (+2)	39 kDa	2	0.47
Lysosomal alpha-glucosidase Tax_id=9606 [Homo sapiens]	sp P10253 GAA	105 kDa	2	0.47
Mucin-5B Tax_id=9606 [Homo sapiens]	sp Q9HC84 MUC5B (+3)	593 kDa	2	0.47
transcript.ENST00000441738				
gene_biotype.protein_coding				
transcript.protein_coding Tax_id=9606 [Homo sapiens]	ens ENSP00000405380 ENSG00000102265 (+5)	11 kDa	2	0.47
Complement component C9 Tax_id=9606 [Homo sapiens]	C9	63 kDa	2	0.59
Pigment epithelium-derived factor Tax_id=9606 [Homo sapiens]	sp P36955 SERPINF1	46 kDa	2	0.59
Carcinoembryonic antigen-related cell adhesion molecule 7 Tax_id=9606 [Homo sapiens]	sp Q14002 CEACAM7 (+3)	29 kDa	2	0.59
Thyroxine-binding globulin Tax_id=9606 [Homo sapiens]	SERPINA7	46 kDa	2	0.59
Desmocollin-2 Tax_id=9606 [Homo sapiens]	sp Q02487 DSC2 (+6)	99 kDa	2 3	0.29
Alpha-2-macroglobulin-like protein 1 Tax_id=9606 [Homo sapiens]	sp A8K2U0 A2ML1 (+2)	160 kDa	2 7	0.027
Corticosteroid binding globulin Tax_id=9606 [Homo sapiens]	sp P08185 SERPINA6	44 kDa	2 7	0.22
Ig gamma-4 chain C region Tax_id=9606 [Homo sapiens]	IGHG4	36 kDa	2 8	0.14
Hemopexin Tax_id=9606 [Homo sapiens]	sp P02790 HPX	51 kDa	3	0.41
FLJ56954, highly similar to Inter-alpha-trypsin inhibitor heavy chain H1 Tax_id=9606 [Homo sapiens]	tr B72539 cDNA	72 kDa	3	0.41
Isoform 2 of Attractin Tax_id=9606 [Homo sapiens]	sp Q75882.2 ATRNL (+4)	138 kDa	3	0.41
Carcinoembryonic antigen-related cell adhesion molecule 5 Tax_id=9606 [Homo sapiens]	sp P06731 CEACAM5 (+4)	74 kDa	4	0.024
protein Tax_id=9606 [Homo sapiens]	tr E7ETN3 Uncharacterized (+1)	123 kDa	4	0.28

Onfatormedin-4 Tax_id=9606 [Homo sapiens]	OLFM4		57 kDa	INF	7	0.073
Serpin B3 Tax_id=9606 [Homo sapiens]	sp P29508 SERPINB3 (+1)		44 kDa	INF		0.56
Fibrinogen alpha chain Tax_id=9606 [Homo sapiens]	sp P02671 FGA		95 kDa	INF		0.56
Prerilycysteine oxidase 1 Tax_id=9606 [Homo sapiens]	sp Q9UHG3 PCYOX1 (+4)		56 kDa	INF		0.018
transfer protein isoform 3 variant (Fragment) Tax_id=9606 [Homo sapiens]	Phospholipid (+3)		54 kDa	INF		0.18
Desmoglein-3 Tax_id=9606 [Homo sapiens]	DSG3		107 kDa	INF		0.1
Peptidoglycan recognition protein 2 Tax_id=9606 [Homo sapiens]	tr B7ZM33 PGLYRP2 (+3)		68 kDa	INF		0.32
Apolipoprotein C-IV Tax_id=9606 [Homo sapiens]	sp P55056 APOC4 (+1)		14 kDa	INF		0.32
Putative uncharacterized protein DESC1 (Fragment) Tax_id=9606 [Homo sapiens]	DESC1 (+6)		47 kDa	INF		0.1
Heparin cofactor 2 Tax_id=9606 [Homo sapiens]	sp P05546 SERPIND1 (+1)		57 kDa	INF		0.32
Isoform 2 of Thrombospondin type 1 domain- containing protein 4 Tax_id=9606 [Homo sapiens]	sp Q6ZMP0-2 THSD4 (+4)		56 kDa	INF		0.32
transcript:ENST00000291804 gene_biotype:protein_coding transcript_biotype:protein_coding Tax_id=9606 [Homo sapiens]	ens ENSP00000375680 ENSG00000167757 (+3)		30 kDa	INF		0.32
Phosphatidylinositol-glycan-specific phospholipase D Tax_id=9606 [Homo sapiens]	GPLD1		91 kDa	INF		0.18
alanine aminopeptidase variant (Fragment) Tax_id=9606 [Homo sapiens]	Membrane (+1)		109 kDa	INF		0.32
transcript:ENST00000264691 gene_biotype:protein_coding transcript_biotype:protein_coding Tax_id=9606 [Homo sapiens]	ens ENSP00000264691 ENSG00000088926 (+3)		18 kDa	INF		0.32
transcript:ENST00000514218 gene_biotype:protein_coding transcript_biotype:protein_coding Tax_id=9606 [Homo sapiens]	ens ENSP00000421626 ENSG00000250722 (+4)		35 kDa	INF		0.32
transcript:ENST00000320216	ens ENSP00000317697 ENSG00000160255		35 kDa	INF		0.56

gene_biotype:protein_coding transcript_biotype:protein_coding Tax_id=9606 [Homo sapiens] transcript:ENST00000544665 gene_biotype:protein_coding transcript_biotype:protein_coding Tax_id=9606 [Homo sapiens]	{+8}				
Phospholipase B-like 1 Tax_id=9606 [Homo sapiens] Putative uncharacterized protein DKFZp686N08236 (Fragment) Tax_id=9606 [Homo sapiens] Prostaglandin-H2 D-isomerase Tax_id=9606 [Homo sapiens]	ens ENSP00000441691 ENSG00000169896 {+3}	125 kDa	INF	0.56	
	PLBD1	63 kDa	INF	0.56	
	DKFZp686N08236 (+8)	13 kDa	INF	0.56	
	sp P41222 PTGDS (+4)	21 kDa	INF	0.68	
Isoform 2 of Inter-alpha-trypsin inhibitor heavy chain H4 Tax_id=9606 [Homo sapiens]	sp Q14624-2 ITH4 (+3)	101 kDa	INF	0.56	
amyloid A protein Tax_id=9606 [Homo sapiens] Isoform 3 of Interleukin-6 receptor subunit beta Tax_id=9606 [Homo sapiens]	tr B2R5G8 Setum	15 kDa	INF	0.56	
Protein AMBP Tax_id=9606 [Homo sapiens] Kallistatin Tax_id=9606 [Homo sapiens] Plasma serine protease inhibitor Tax_id=9606 [Homo sapiens]	sp P40189-3 IL6ST (+2) sp P02760 AMBP SERPINA4	95 kDa 39 kDa 48 kDa	INF INF INF	0.56 0.56 0.56	
Putative uncharacterized protein DKFZp686O1553 (Fragment) Tax_id=9606 [Homo sapiens] Complement C1q subcomponent subunit A Tax_id=9606 [Homo sapiens] Transmembrane protease serine 118 Tax_id=9606 [Homo sapiens]	sp P05154 SERPINA5 (+7) DKFZp686O1553 (+9) C1QA (+1)	45 kDa 54 kDa 26 kDa	INF INF INF	0.56 0.56 0.56	
Cholinesterase Tax_id=9606 [Homo sapiens] Isoform LMW of Kininogen-1 Tax_id=9606 [Homo sapiens] Putative uncharacterized protein DKFZp779H1622 (Fragment) Tax_id=9606 [Homo sapiens]	TMPS511B sp P06276 BCHE (+3) sp P01042-2 KNG1 (+2) DKFZp779H1622 (+1)	45 kDa 67 kDa 47 kDa 35 kDa	INF INF INF INF	0.56 0.32 0.32 0.56	

glycoprotein variant (Fragment) Tax_id=9606 [Homo sapiens]	Oviduct-specific (+5)	82 kDa	INF	0.56
Thrombospondin-1 Tax_id=9606 [Homo sapiens]	THBS1 (+2)	129 kDa	INF	0.56
Properdin Tax_id=9606 [Homo sapiens]	CFP	51 kDa	INF	0.56
Coagulation factor XIII B chain Tax_id=9606 [Homo sapiens]	F13B	75 kDa	INF	0.56
Vasorin Tax_id=9606 [Homo sapiens]	VASN	71 kDa	INF	0.56
transcript:ENST00000279452				
gene_biotype:protein_coding				
transcript_biotype:protein_coding Tax_id=9606 [Homo sapiens]	ens ENSP00000279452 ENSG0000026508 (+39)	30 kDa	INF	0.56
Protein C20orf3 (Fragment) Tax_id=9606 [Homo sapiens]	tr H0Y512 C20orf3 (+4)	45 kDa	INF	0.56

Table 22

ConcavalinA Proteomics Method 2 Early Secretory Fertile v Early Secretory Infertile Results

Identified Proteins (116)	Accession Number	Molecular Weight	Fold Change	p value
Tax_id=9606 [Homo sapiens]	Glycodelin (+1)	16 kDa	0	0.00069
sulfate proteoglycan 2 (Versican) variant (Fragment) Tax_id=9606 [Homo sapiens]	Chondroitin (+9)	372 kDa	0	0.0016
transcript:ENST00000416334				
gene_biotype:protein_coding				
transcript_biotype:protein_coding Tax_id=9606 [Homo sapiens]	ens ENSP00000414674 ENSG00000135318 (+8)	26 kDa	0	0.09
Isoform Somatic-2 of Angiotensin-converting	sp P12821-2 ACE (+2)	130 kDa	0	0.04

enzyme Tax_id=9606 [Homo sapiens]				
amidohydrolase (Acid ceramidase) 1 preproprotein isoform a variant (Fragment) Tax_id=9606 [Homo sapiens]	N-acylsphingosine (+9)	44 kDa	0	0.45
Legumain Tax_id=9606 [Homo sapiens]	sp Q99538 LGMN (+8)	49 kDa	0	0.45
Alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase 1 Tax_id=9606 [Homo sapiens]	sp Q9NSC7 STGGALNAC1 (+2)	68 kDa	0	0.45
transcript:ENST00000548954				
gene_biotype:protein_coding				
transcript_biotype:protein_coding Tax_id=9606 [Homo sapiens]	ens ENSP00000146799 ENSG00000135678 (+7)	28 kDa	0	0.2
Isoform 3 of CD109 antigen Tax_id=9606 [Homo sapiens]	sp Q6VHK3-3 CD109 (+2)	73 kDa	0	0.2
Fibrocystin-1 Tax_id=9606 [Homo sapiens]	sp Q86WH1 PKHD111	460 kDa	0	0.2
Carrier family 6, member 8 variant (Fragment) Tax_id=9606 [Homo sapiens]	PPGB (+8)	56 kDa	0	0.2
Transcobalamin-1 Tax_id=9606 [Homo sapiens]	TCN1	47 kDa	0	0.45
transcript:ENST00000429820				
gene_biotype:protein_coding				
transcript_biotype:protein_coding Tax_id=9606 [Homo sapiens]	ens ENSP00000388826 ENSG00000151655 (+5)	27 kDa	0.1	0.24
Putative uncharacterized protein DKFZp686O12165 (Fragment) Tax_id=9606 [Homo sapiens]	DKFZp686O12165 (+18)	240 kDa	0.2	0.4
Serum paraoxonase/arylesterase 1 Tax_id=9606 [Homo sapiens]	sp P27169 PON1 (+2)	39 kDa	0.2	0.4
Isoform Sap-mu-6 of Proactivator polypeptide Tax_id=9606 [Homo sapiens]	sp P07602-2 PSAP (+10)	58 kDa	0.2	0.24
Complement C3 Tax_id=9606 [Homo sapiens]	sp P01024 C3	187 kDa	0.3	0.26
transcript:ENST000003480947				
gene_biotype:protein_coding				
transcript_biotype:nonsense_mediated_decay Tax_id=9606 [Homo sapiens]	ens ENSP00000370334 ENSG00000181143 (+5)	133 kDa	0.3	0.067

Alpha-1-acid glycoprotein 2 [Tax_id=9606 [Homo sapiens]	ORM2		23 kDa	0.3	0.42
Apolipoprotein D [Tax_id=9606 [Homo sapiens]	sp P05090 APOD (+2)		21 kDa	0.3	0.25
transcript:ENST00000576830					
gene_biotype:protein_coding					
transcript_biotype:protein_coding [Tax_id=9606 [Homo sapiens]	ens ENSP00000460219 ENSG000000129214 (+10)		28 kDa	0.3	0.42
transcript:ENST00000438213					
gene_biotype:protein_coding					
transcript_biotype:protein_coding [Tax_id=9606 [Homo sapiens]	ens ENSP00000415036 ENSG000000117984 (+5)		32 kDa	0.3	0.42
amyloid A protein [Tax_id=9606 [Homo sapiens]	tr B2R5G8 Serum		15 kDa	0.3	0.42
Kininogen 1, isoform CRA_b [Tax_id=9606 [Homo sapiens]	tr B4E1C2 KNG1 (+1)		71 kDa	0.4	0.39
transcript:ENST00000264690					
gene_biotype:protein_coding					
transcript_biotype:protein_coding [Tax_id=9606 [Homo sapiens]	ens ENSP00000264690 ENSG000000164344 (+7)		71 kDa	0.4	0.4
Galectin-3-binding protein [Tax_id=9606 [Homo sapiens]	sp Q08380 LGALS3BP (+2)		65 kDa	0.5	0.086
transcript:ENST00000572672					
gene_biotype:protein_coding					
transcript_biotype:protein_coding [Tax_id=9606 [Homo sapiens]	ens ENSP00000461873 ENSG000000262062 (+3)		13 kDa	0.5	0.7
Polymeric immunoglobulin receptor [Tax_id=9606 [Homo sapiens]	PIGR		22 kDa	2.3	0.18
Ig gamma-4 chain C region [Tax_id=9606 [Homo sapiens]	IGHG4		36 kDa	2.3	0.36
IgGc-binding protein [Tax_id=9606 [Homo sapiens]	FCGBP		568 kDa	2.4	0.26
Pigment epithelium-derived factor [Tax_id=9606 [Homo sapiens]	sp P36955 SERPINF1		46 kDa	2.4	0.58
Lysosomal alpha-glucosidase [Tax_id=9606 [Homo sapiens]	sp P10253 GAA		105 kDa	2.4	0.58

alanine aminopeptidase variant (Fragment) Tax_id=9606 [Homo sapiens]	Membrane (+1)	109 kDa	2.4	0.58
Putative uncharacterized protein DKFZp686N08236 (Fragment) Tax_id=9606 [Homo sapiens]	DKFZp686N08236 (+8)	13 kDa	2.4	0
Isoform 10 of Carcinoembryonic antigen-related cell adhesion molecule 1 Tax_id=9606 [Homo sapiens]	sp P13688.10 CEACAM1 (+6)	49 kDa	2.6	0.44
FLJ56954, highly similar to Inter-alpha trypsin inhibitor heavy chain H1 Tax_id=9606 [Homo sapiens]	tr B7Z539 cDNA	72 kDa	3.1	0.39
Carcinoembryonic antigen-related cell adhesion molecule 5 Tax_id=9606 [Homo sapiens]	sp P06731 CEACAM5 (+4)	74 kDa	4.8	0.014
FLJ36533 fs. clone TRACH200428, highly similar to Lactotransferrin (EC 3.4.21.-) (Fragment) Tax_id=9606 [Homo sapiens]	tr B3KSL2 cDNA (+1)	76 kDa	4.8	0.39
Mucin-5B Tax_id=9606 [Homo sapiens]	sp Q9HC84 MUC5B (+3)	593 kDa	6.6	0.26
Fibrinogen alpha chain Tax_id=9606 [Homo sapiens]	sp P02671 FGA	95 kDa	INF	0.55
Hemupexin Tax_id=9606 [Homo sapiens]	sp P02790 HPX	51 kDa	INF	0.55
Complement factor B Tax_id=9606 [Homo sapiens]	tr B4F174 CFB (+1)	140 kDa	INF	0.092
Desmoglein-3 Tax_id=9606 [Homo sapiens]	DSG3	107 kDa	INF	0.092
Carcinoembryonic antigen-related cell adhesion molecule 7 Tax_id=9606 [Homo sapiens]	sp Q14002 CEACAM7 (+3)	29 kDa	INF	0.3
Transmembrane protease serine 11E Tax_id=9606 [Homo sapiens]	sp Q9UL52 TMPRSS11E (+6)	47 kDa	INF	0.092
Prenylcysteine oxidase 1 Tax_id=9606 [Homo sapiens]	sp Q9UHG3 PCYOX1 (+4)	56 kDa	INF	0.092
Isoform 2 of Thrombospondin type-1 domain- containing protein 4 Tax_id=9606 [Homo sapiens]	sp Q6ZMP0-2 THSD4 (+4)	56 kDa	INF	0.3
Heparin cofactor 2 Tax_id=9606 [Homo sapiens]	sp P05546 SERPIND1 (+1)	57 kDa	INF	0.3
Phosphatidylinositol-glycan-specific phospholipase D Tax_id=9606 [Homo sapiens]	GPIID1	91 kDa	INF	0.3
Isoform 3 of Interleukin-6 receptor subunit beta Tax_id=9606 [Homo sapiens]	sp P40189-3 IL6ST (+2)	95 kDa	INF	0.55

transcript:ENST00000391804	ens ENSP00000375680 ENSG00000167757			
gene_biotype:protein_coding	(+3)		30 kDa	INF
transcript_biotype:protein_coding				
[Homo sapiens]				
Phospholipase B-like 1 Tax_id=9606 [Homo sapiens]	PLBD1		63 kDa	INF
Transmembrane protease serine 11B Tax_id=9606	TMPS511B		45 kDa	INF
[Homo sapiens]				
Cholinesterase Tax_id=9606 [Homo sapiens]	sp P06276 BCHE (+3)		67 kDa	INF
Isoform LMW of Kininogen 1 Tax_id=9606 [Homo sapiens]	sp P01042.2 KNG1 (+2)		47 kDa	INF
Kallistatin Tax_id=9606 [Homo sapiens]	SERPINA4		48 kDa	INF
Putative uncharacterized protein DKFZp79H1622 (Fragment) Tax_id=9606 [Homo sapiens]	DKFZp79H1622 (+1)		35 kDa	INF
Complement C1q subcomponent subunit A Tax_id=9606 [Homo sapiens]	C1QA (+1)		26 kDa	INF
Coagulation factor XIII B chain Tax_id=9606 [Homo sapiens]	F13B		75 kDa	INF
Vasorin Tax_id=9606 [Homo sapiens]	VASN		71 kDa	INF
transcript:ENST00000279452				
gene_biotype:protein_coding	ens ENSP00000279452 ENSG00000026508		30 kDa	INF
transcript_biotype:protein_coding	(+39)			
[Homo sapiens]				
transcript:ENST00000451442				
gene_biotype:protein_coding	ens ENSP00000395874 ENSG00000101474		45 kDa	INF
transcript_biotype:protein_coding	(+4)			
[Homo sapiens]				
transcript:ENST00000514218				
gene_biotype:protein_coding	ens ENSP00000421626 ENSG00000250722		35 kDa	INF
transcript_biotype:protein_coding	(+4)			
[Homo sapiens]				
Isoform 2 of Extracellular matrix protein 1 Tax_id=9606 [Homo sapiens]	sp Q16610-2 ECM1 (+2)		46 kDa	INF
Isoform 2 of N-acetylglucosyl-L-alanine amidase	sp Q96PD5.2 PGLYRP2 (+3)		68 kDa	INF

Tax_Id=9606 [Homo sapiens]			
Properdin Tax_Id=9606 [Homo sapiens]	CFP	51 kDa	INF 0.55
Putative uncharacterized protein DKFZp686O1553 (Fragment) Tax_Id=9606 [Homo sapiens]	DKFZp686O1553 (+9)	54 kDa	INF 0.55
Protein AMBP Tax_Id=9606 [Homo sapiens]	sp P02760 AMBP (+1)	39 kDa	INF 0.55

Table 23

Concavalin A Proteomics Method 2 Mid Secretory Fertile v Mid Secretory Infertile Results

Identified Proteins (96)	Accession Number	Molecular Weight	Fold Change	P-Value
Complement factor B Tax_Id=9606 [Homo sapiens]	tr B4E1Z4 CFB (+2)	140 kDa	0	0.42
Golgi membrane protein 1 Tax_Id=9606 [Homo sapiens]	sp Q8NEJ4 GOLM1 (-3)	45 kDa	0	0.17
3 type VI collagen isoform 5 variant (Fragment) Tax_Id=9606 [Homo sapiens]	Alpha (+9)	182 kDa	0	0.17
Isoform 2 of Extracellular matrix protein 1 Tax_Id=9606 [Homo sapiens]	sp Q31661 E2 ECM1 (+2)	46 kDa	0	0.42
Zinc-alpha-2-glycoprotein Tax_Id=9606 [Homo sapiens]	sp P25311 AZGP1 (+2)	34 kDa	0	0.17
BPI fold-containing family B member 1 Tax_Id=9606 [Homo sapiens]	BP1FB1 (+1)	52 kDa	0	0.42
transcript:ENST000002567027				
gene_biotype:protein_coding				
transcript_biotype:nonsense_mediated_decay Tax_Id=9606 [Homo sapiens]	ens ENSP00000457521 ENSG00000213614 (+7)	36 kDa	0	0.42
Mucin 5B Tax_Id=9606 [Homo sapiens]	sp Q5HCB4 MLUC5B (+3)	593 kDa	0	0.42
HP protein Tax_Id=9606 [Homo sapiens]	tr O6PEJ8 HP (+1)	25 kDa	0	0.0001
transcript:ENST000002546291				
gene_biotype:protein_coding				
transcript_biotype:protein_coding Tax_Id=9606	ens ENSP00000446530 ENSG000000011465 (+6)	19 kDa	0	0.42

[Homo sapiens] Neutrophil gelatinase-associated lipocalin Tax_id=9606 [Homo sapiens]	NGAL (+4)	23 kDa	0.2	0.35
Isoform H14 of Myeloperoxidase [Homo sapiens]	sp P05164-2 MPO (+2)	73 kDa	0.2	0.2
Isoform 10 of Carcinoembryonic antigen-related cell adhesion molecule 1 [Tax_id=9606 [Homo sapiens]	sp P13688-10 CEACAM1 (+6)	49 kDa	0.2	0.35
Azuricidin [Tax_id=9606 [Homo sapiens]	sp P20160 AZU1 (+1)	27 kDa	0.2	0.38
Neutrophil elastase [Fragment] [Tax_id=9606 [Homo sapiens]	ELA2 (+1)	20 kDa	0.2	0.38
Desmocollin-2 [Tax_id=9606 [Homo sapiens]	sp Q02487 DSC2 (+8)	99 kDa	0.2	0.66
transcript ENST00000438213 gene_biotype:protein_coding transcript_biotype:protein_coding Tax_id=9606 [Homo sapiens]	ens ENSP000000415036 ENSG000000117984 (+5)	32 kDa	0.2	0.66
IgGf-binding protein [Tax_id=9606 [Homo sapiens]	FCG8P	568 kDa	0.3	0.0078
Cathepsin L2 [Tax_id=9606 [Homo sapiens]	CTSL2 (+1)	37 kDa	0.3	0.56
EL151523, highly similar to Inter-alpha-trypsin inhibitor heavy chain H1 [Tax_id=9606 [Homo sapiens]	tr B7Z558 tDNA (+3)	86 kDa	0.4	0.66
transcript ENST00000534950 gene_biotype:protein_coding transcript_biotype:protein_coding Tax_id=9606 [Homo sapiens]	ens ENSP000000439180 ENSG000000139178 (+3)	21 kDa	0.4	0.66
Complement component C48 (t hildn blood group) [Tax_id=9606 [Homo sapiens]	C4B-1 (+20)	192 kDa	0.5	0.52
Carcinoembryonic antigen-related cell adhesion molecule 5 [Tax_id=9606 [Homo sapiens]	sp P06731 CEACAM5 (+4)	74 kDa	0.5	0.44
Biotinidase [Tax_id=9606 [Homo sapiens]	sp P43251 BITD (+6)	60 kDa	0.5	0.35
Lysosomal alpha-glucosidase [Tax_id=9606 [Homo sapiens]	sp P10253 GAA	105 kDa	0.5	0.56
plasma protease C1 inhibitor [Tax_id=9606 [Homo sapiens]	sp P05155 SERPING1 (+4)	54 kDa	2	0.62

transcript:ENST00000576830					
gene biotype:protein coding					
transcript_biotype:protein_coding	Tax_id=9606				
[Homo sapiens]					
transcript:ENST00000264690					
gene biotype:protein coding					
transcript_biotype:protein_coding	Tax_id=9606				
[Homo sapiens]					
Leucine-rich alpha-2-glycoprotein	Tax_id=9606 [Homo sapiens]				
HMFT1766 (+2)					
transcript:ENST00000453131					
gene biotype:protein coding					
transcript_biotype:protein_coding	Tax_id=9606				
[Homo sapiens]					
Vitronectin	Tax_id=9606 [Homo sapiens]				
sp P04004 VTN (+1)					
Complement C3	Tax_id=9606 [Homo sapiens]				
sp P01024 C3					
Olfactomedin 4	Tax_id=9606 [Homo sapiens]				
OLF4					
Fibrinogen beta chain	Tax_id=9606 [Homo sapiens]				
sp P02675 FGB (+3)					
Inter-alpha-trypsin inhibitor heavy chain H2	Tax_id=9606 [Homo sapiens]				
sp P19823 ITH2 (+3)					
Fibronectin-1	Tax_id=9606 [Homo sapiens]				
sp Q86W11 PKHD1L1					
Corticosteroid-binding globulin	Tax_id=9606 [Homo sapiens]				
sp P08185 SERPINAG					
Serpin B3	Tax_id=9606 [Homo sapiens]				
sp P29508 SERPINB3 (+1)					
Isoform 2 of N-acetylmuramoyl-L-alanine amidase					
Tax_id=9606 [Homo sapiens]					
sp Q96PD5-2 PGLYRP2 (+3)					
Serum paraoxonase/arylesterase 1	Tax_id=9606 [Homo sapiens]				
sp P27169 PON1 (+2)					
Apolipoprotein C-IV	Tax_id=9606 [Homo sapiens]				
sp P55056 APOC4 (+1)					
Preylkysteine oxidase 1	Tax_id=9606 [Homo sapiens]				
sp Q9UHG3 PCYOX1 (+4)					
Complement component C9	Tax_id=9606 [Homo sapiens]				
C9					

Phospholipid transfer protein	Tax_id=9606 [Homo sapiens]	sp P55058 PLTP (+3)	54 kDa	INF	0.58
transcript:ENST00000514218					
gene_biotype:protein_coding					
transcript_biotype:protein_coding	Tax_id=9606 [Homo sapiens]	ens ENSP000000421G26 ENSG000000250722 (+4)	35 kDa	INF	0.58
transcript_biotype:protein_coding	Tax_id=9606 [Homo sapiens]	sp P01008 SERPINC1	52 kDa	INF	0.58
Antithrombin-III	Tax_id=9606 [Homo sapiens]	sp P11279 LAMP1 (+3)	43 kDa	INF	0.34
Lysosome-associated membrane glycoprotein 1	Tax_id=9606 [Homo sapiens]	sp Q99538 LGMN (+8)	49 kDa	INF	0.34
Legumain	Tax_id=9606 [Homo sapiens]	D5G3	107 kDa	INF	0.58
Desmoglein-3	Tax_id=9606 [Homo sapiens]	GPLD1	91 kDa	INF	0.58
Phosphatidylinositol-glycan-specific phospholipase D	Tax_id=9606 [Homo sapiens]	Glycodelin (+2)	16 kDa	INF	0.58
Tax_id=9606 [Homo sapiens]		Oviduct-specific (+5)	82 kDa	INF	0.58
glycoprotein variant (Fragment)	Tax_id=9606 [Homo sapiens]				
amidohydrolase (Acid ceramidase) 1 preproprotein	isoform a variant (Fragment)	N-acylsphingosine (+9)	44 kDa	INF	0.58
isoform a variant (Fragment)	Tax_id=9606 [Homo sapiens]	PLBD1	63 kDa	INF	0.58
Phospholipase B-like 1	Tax_id=9606 [Homo sapiens]	SERPINA4	48 kDa	INF	0.58
Kallistatin	Tax_id=9606 [Homo sapiens]				
transcript:ENST00000573763					
gene_biotype:protein_coding					
transcript_biotype:protein_coding	Tax_id=9606 [Homo sapiens]	ens ENSP000000461405 ENSG000000132386 (+3)	16 kDa	INF	0.58
transcript_biotype:protein_coding	Tax_id=9606 [Homo sapiens]	sp Q9UBD6 RHC6 (+8)	53 kDa	INF	0.58
Ammonium transporter Rh type C	Tax_id=9606 [Homo sapiens]	sp Q9UL52 TMPRSS11E (+6)	47 kDa	INF	0.58
Transmembrane protease serine 11E	Tax_id=9606 [Homo sapiens]	THBS1 (+2)	129 kDa	INF	0.58
Thrombospondin-1	Tax_id=9606 [Homo sapiens]				
Isoform B of Proteoglycan 4	Tax_id=9606 [Homo sapiens]	sp Q92954-2 PRG4 (+7)	146 kDa	INF	0.58

isoform OA3-293 of Leukocyte surface antigen CD47					
Tax_id=9606 [Homo sapiens]					
Thyroxine-binding globulin Tax_id=9606 [Homo sapiens]	sp Q08722-2 CD47 (+3)	31 kDa	INF	0.58	
transcript:ENST00000264691	SERPINA7	46 kDa	INF	0.58	
gene_biotype:protein_coding					
transcript_biotype:protein_coding Tax_id=9606 [Homo sapiens]	ens ENSP00000264691 ENSG000000088926 (+3)	18 kDa	INF	0.58	
T2 Tax_id=9606 [Homo sapiens]	Ribonuclease (+8)	35 kDa	INF	0.58	

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CLAIMS

1. An assay to identify an endometrial protein glycoform biomarker in uterine lavage from a female mammal which is indicative of a level of receptivity of an endometrium for a likely successful or likely unsuccessful embryo implantation, said assay comprising selecting an endometrial protein having a glycoform which specifically binds to a lectin forming an endometrial protein-lectin binding pair capturing the protein on a solid support using an immobilized primary antibody specific for said protein and then detecting the glycoform of the captured protein using the lectin specific for said glycoform wherein the lectin is labeled with a reporter molecule capable of being detected or binding a labeled antibody specific for said lectin which antibody is labeled with a reporter molecule capable of being detected.
2. The assay of Claim 1 wherein the female mammal is a human female.
3. The assay of Claim 2 wherein the protein-lectin pair discriminates between a fertile and an infertile human female during mid-secretory phase.
4. The assay of Claim 2 wherein the protein-lectin pair discriminates likely pregnancy outcome in a human female undergoing assisted reproduction treatment.
5. The assay of any one of Claims 1 to 4 wherein the lectin is selected from the list in Table 6.
6. The assay of any one of Claims 1 to 4 wherein the protein-lectin pair is selected from the list in Table 3.
7. The assay of any one of Claims 1 to 4 wherein the protein-lectin pair is selected from the list in Table 4.
8. Use of a endometrial protein-lectin binding pair, wherein the lectin binds to a specific glycoform of the endometrial protein in the manufacture of an assay to detect uterine receptivity or otherwise for an embryo implant in a female mammal.
9. Use of Claim 8 wherein the female mammal is a human female.

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10. Use of Claim 9 wherein the protein-lectin pair discriminates between a fertile and an infertile human female during mid-secretory phase.
11. Use of Claim 9 wherein the protein-lectin pair discriminates likely pregnancy outcome in a human female undergoing assisted reproduction treatment.
12. Use of any one of Claims 8 to 11 wherein the lectin is selected from the list in Table 6.
13. Use of any one of Claims 8 to 11 wherein the protein-lectin pair is selected from the list in Table 3.
14. Use of any one of Claims 8 to 11 wherein the protein-lectin pair is selected from the list in Table 5.
15. A method of assisted reproduction in a female mammal said method comprising selecting an endometrial protein having a glycoform which specifically binds to a lectin forming a protein-lectin pair, capturing the protein on a solid support using an immobilized primary antibody specific for said protein and then detecting the glycoform of the captured protein using the lectin specific for said glycoform wherein the lectin is labeled with a reporter molecule capable of being detected or binding a labeled antibody specific for said lectin which antibody is labeled with a reporter molecule capable of being detected wherein the presence or absence of the protein-lectin pair is indicative of whether an embryo implant should proceed.
16. The method of Claim 15 wherein the female mammal is a human.
17. The method of Claim 16 wherein the protein-lectin pair discriminates between a fertile and an infertile human female during mid-secretory phase.
18. The method of Claim 16 wherein the protein-lectin pair discriminate likely pregnancy outcome in a human female undergoing assisted reproduction treatment.
19. The method of any one of Claims 15 to 18 wherein the lectin is selected from the list in Table 6.

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20. The method of any one of Claims 15 to 18 wherein the protein-lectin pair is selected from the list in Table 3.
21. The method of any one of Claims 15 to 18 wherein the protein-lectin pair is selected from the list in Table 4.
22. A method of determining a female mammal that is likely to fall pregnant, the method comprising selecting an endometrial protein having a glycoform which specifically binds to a lectin forming a protein-lectin pair, capturing the protein on a solid support using an immobilized primary antibody specific for the protein and then detecting the glycoform of the captured protein using the lectin specific for the glycoform wherein the lectin is labeled with a reporter molecule capable of being detected or binding a labeled antibody specific for the lectin which antibody is labeled with a reporter molecule capable of being detected wherein the presence or absence or level of the protein-lectin pair is indicative of a receptive endometrial for pregnancy.
23. A method of Claim 22 wherein the female mammal is a human.
24. A method of Claim 22 wherein the protein-lectin pair discriminates between a fertile and an infertile human female during mid-secretory phase.
25. A method of Claim 22 wherein the protein-lectin pair discriminate likely pregnancy outcome in a human female undergoing assisted reproduction treatment.
26. A method of any one of Claims 15 to 22 wherein the lectin is selected from the list in Table 6.
27. A method of any one of Claims 15 to 22 wherein the protein-lectin pair is selected from the list in Table 3.
28. A method of any one of Claims 15 to 22 wherein the protein-lectin pair is selected from the list in Table 5.

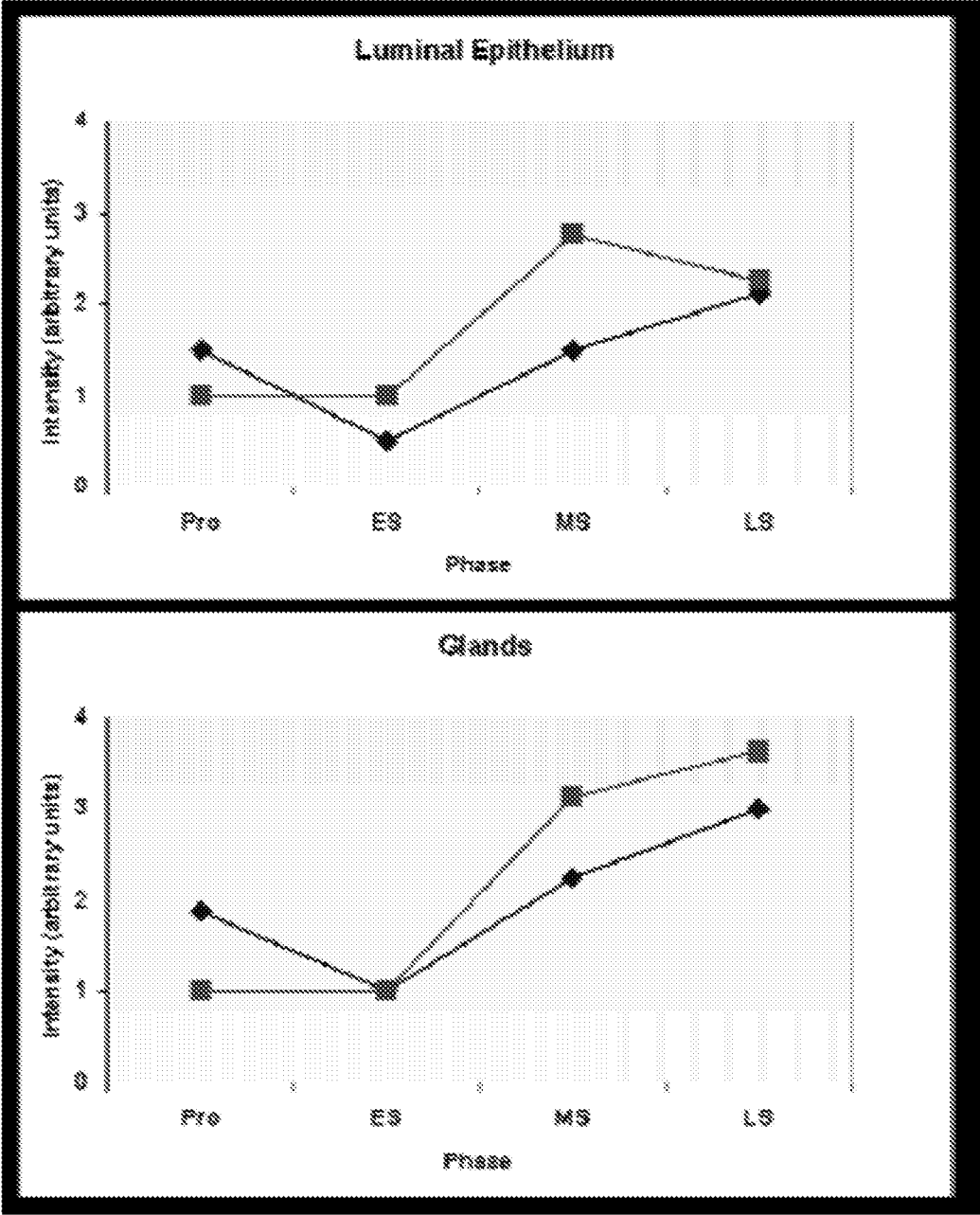


Figure 1

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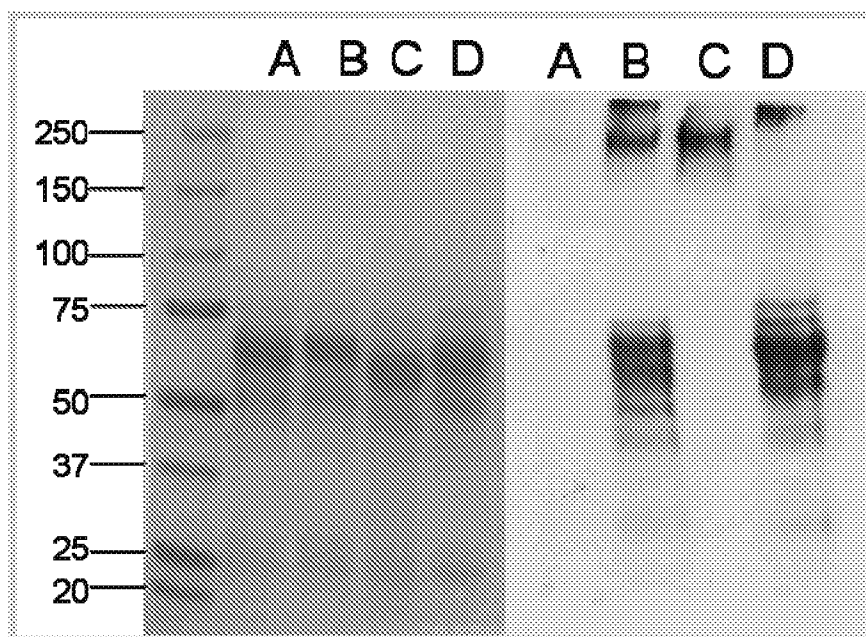


Figure 2

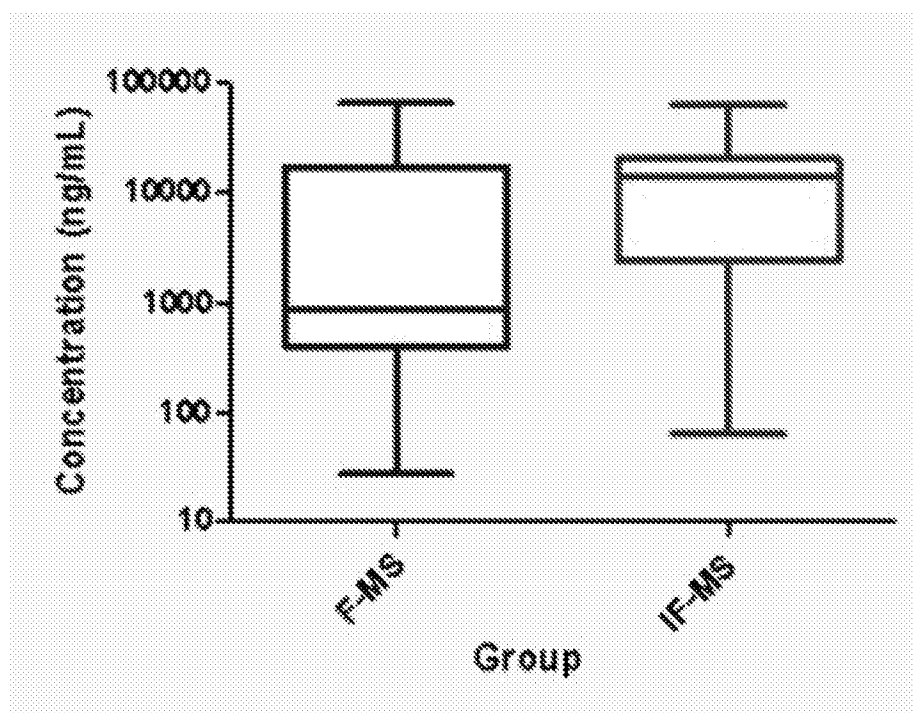
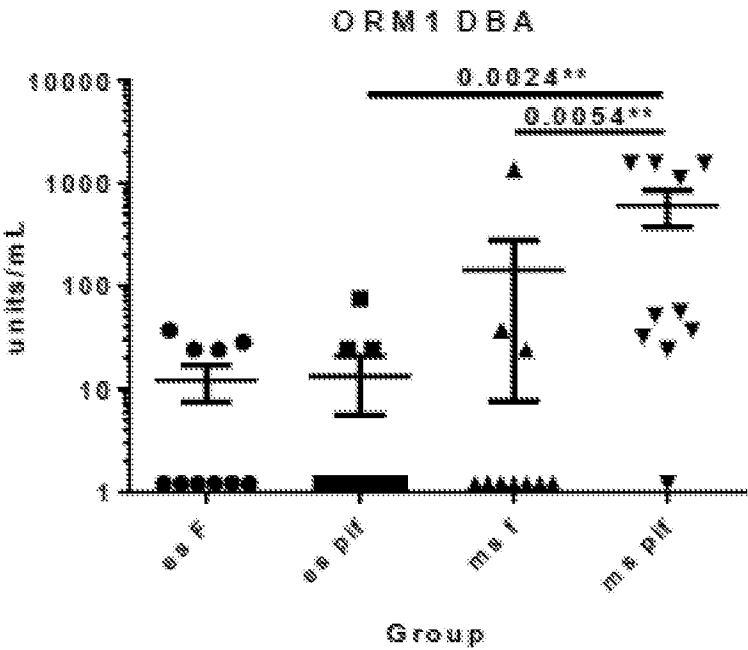


Figure 3

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A



B

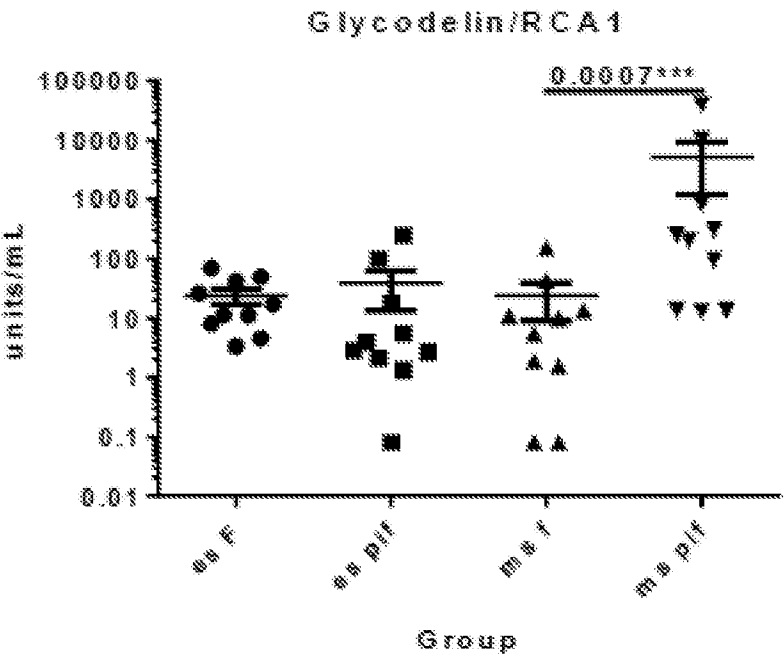


Figure 4A and B

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C

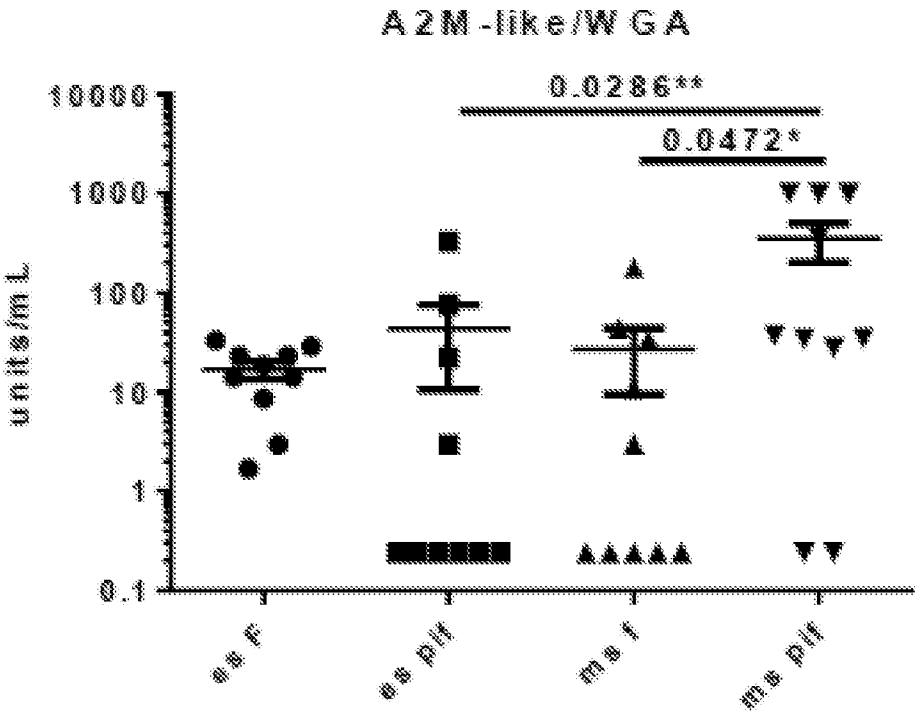
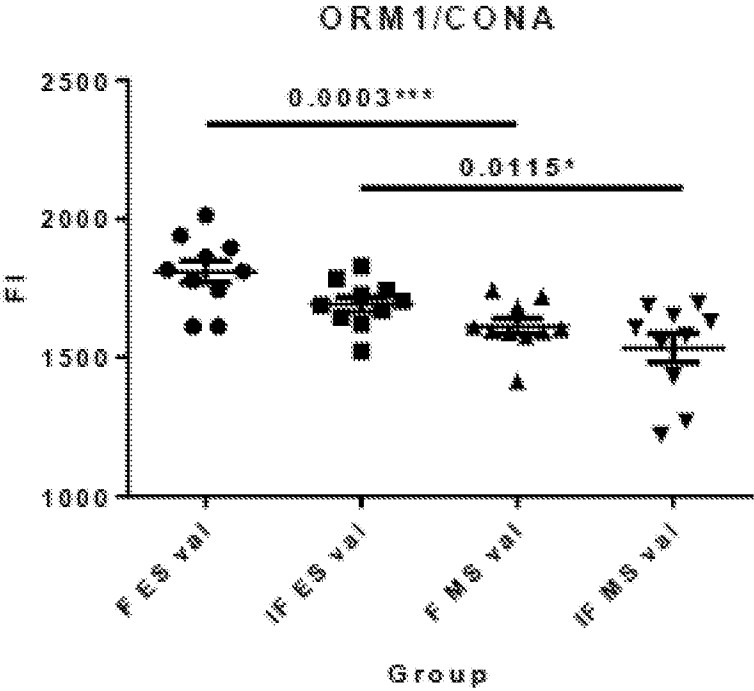


Figure 4C

A



B

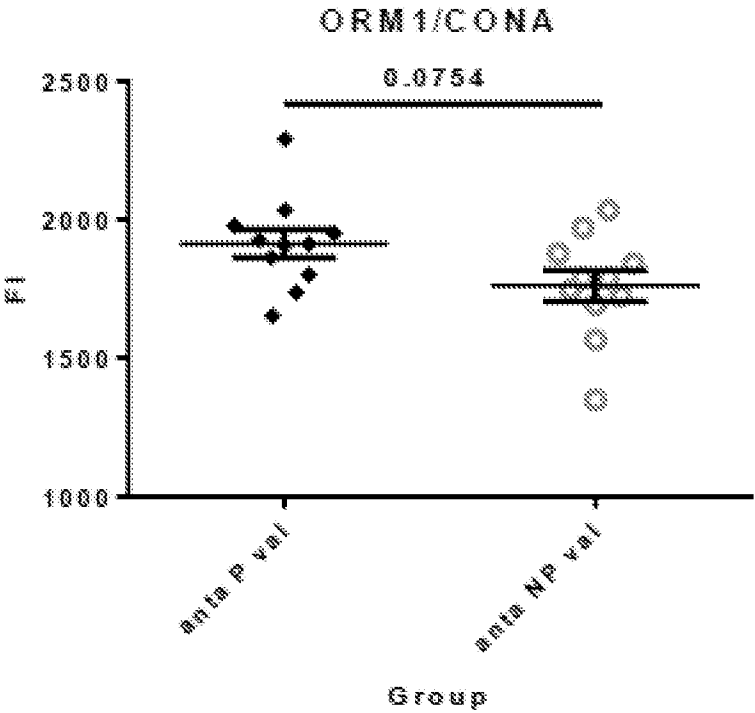


Figure 5A and B

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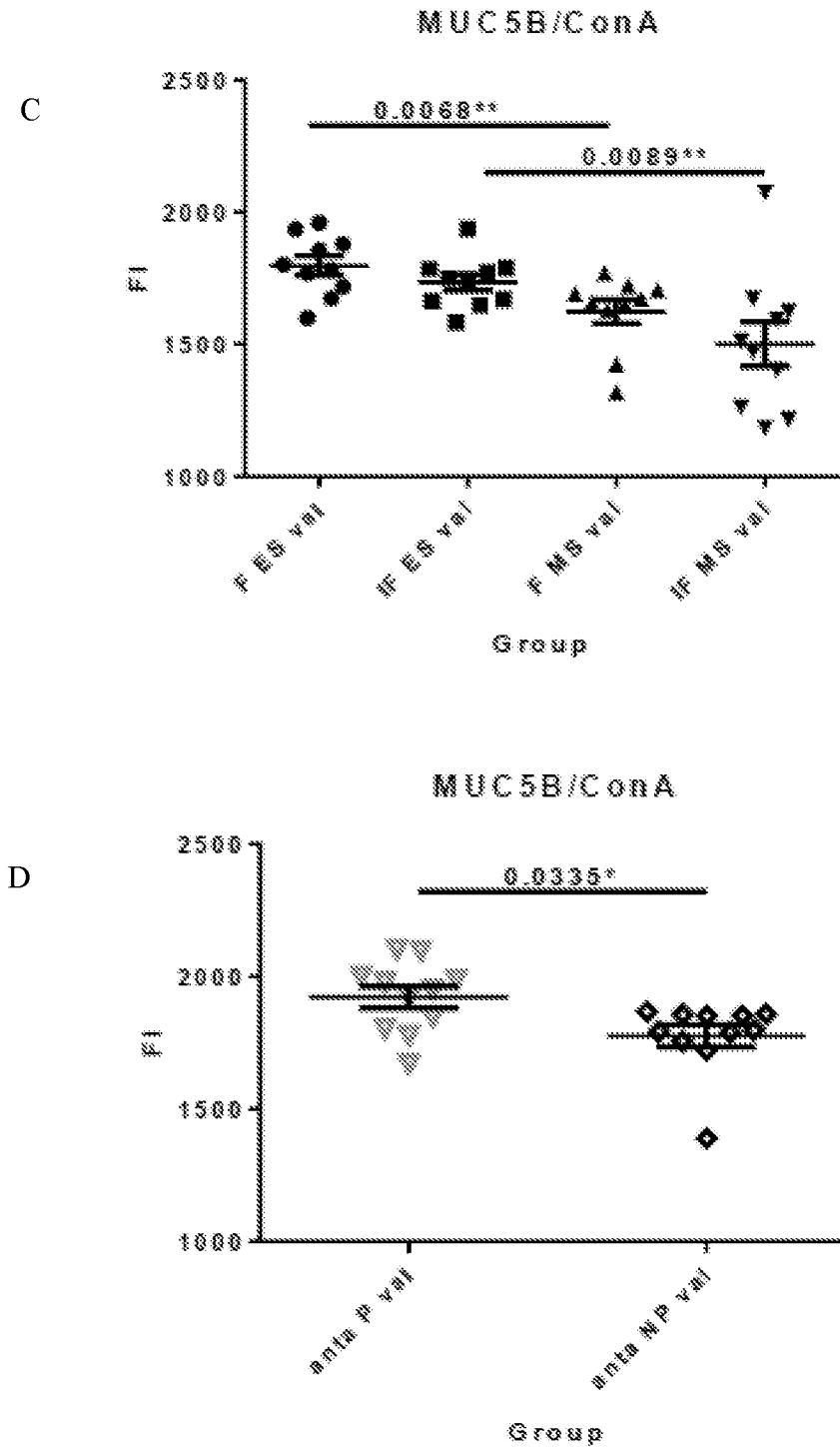


Figure 5C and D

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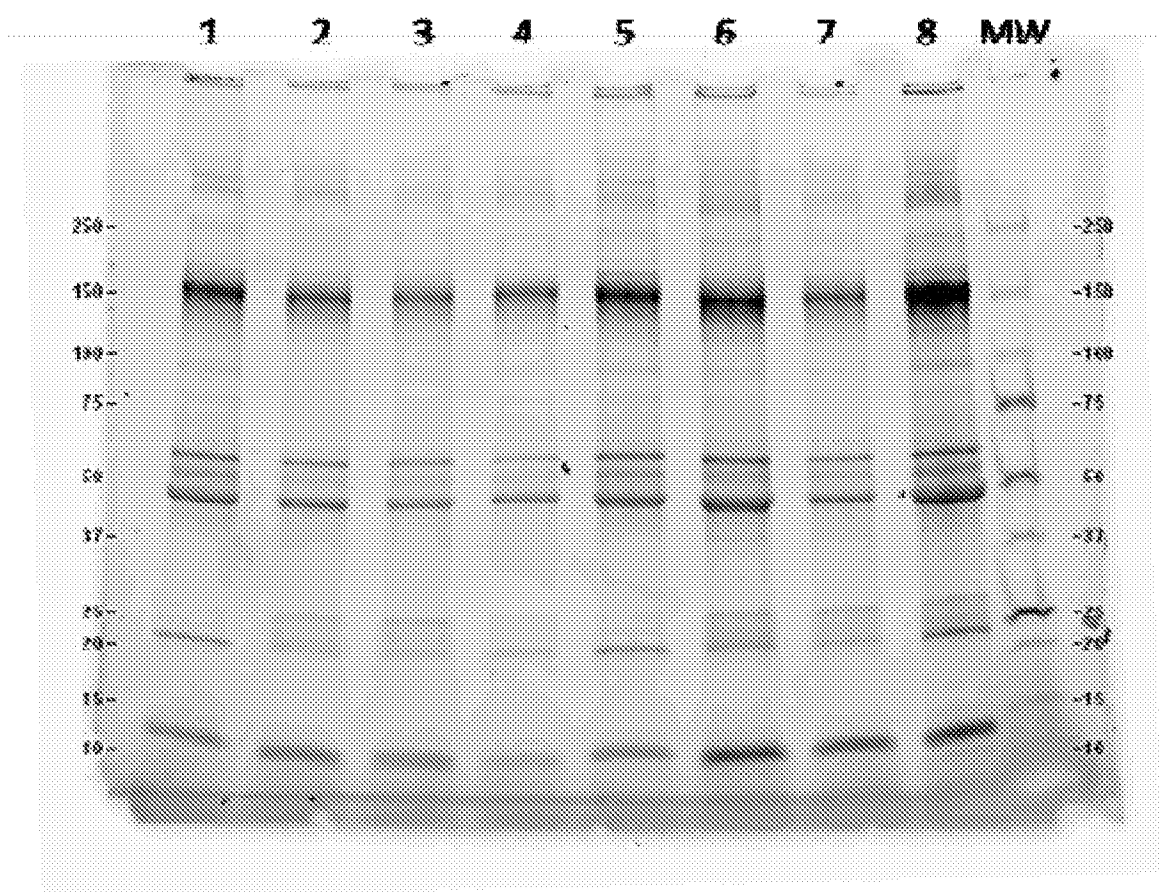


Figure 6

INTERNATIONAL SEARCH REPORT

 International application No.
PCT/AU2016/050648

A. CLASSIFICATION OF SUBJECT MATTER

G01N 33/53 (2006.01)

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)

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)

Databases: WPIAP, EPODOC, CAPLUS, MEDLINE, EMBASE, BIOSIS; Keywords: glycoform, lectin, DBA, ConA, WGA, ricin agglutinin, glycodelin, PAEP, PP14, A2ML1, TAFI, CPB2, MUC5B, implantation, infertility, uterine lavage, endometrial receptivity. Applicant/inventor search: Google Scholar, AUSPAT, Patentscope

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"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
"E"	earlier application or patent but published on or after the international filing date	"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
"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)	"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
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 8 September 2016	Date of mailing of the international search report 08 September 2016
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au	Authorised officer Daniel Sheahan AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61 2 6283 7969

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2016/050648
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014/0057286 A1 (SYSMEX CORPORATION et al.) 27 February 2014 Abstract, [0044]-[0045], [0071], [0074], [0076] [0123]-[0126], [0197], Example 1, Figure 8	8-14
X	US 2012/0190576 A1 (NARIMATSU et al.) 26 July 2012 Abstract, [0106]-[0111], [0249], Tables 3, 4, 6	8-14
Y	SKRZYPCZAK, J. et al., "[Is glycodelin an important marker of endometrial receptivity?]", Ginekologia Polska, 2005, Vol. 76, No. 10, pages 770-781 English summary	1-7, 15-28
X Y	CHIU, P.C.N. et al., "Zona-binding inhibitory factor-1 from human follicular fluid is an isoform of glycodelin", Biology of Reproduction, 2003, Vol. 69, pages 365-372 page 366, paragraph 10; Table 1 as above	8-14 1-7, 15-28
X Y	KOISTINEN, H. et al., "Glycodelin from seminal plasma is a differentially glycosylated form of contraceptive glycodelin-A", Molecular Human Reproduction, 1996, Vol. 2, No. 10, pages 759-765 page 761, paragraph 5; Table I as above	8-11 1-4, 15-18, 22-25
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INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/AU2016/050648	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
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