Trabajo de Revisión | Toxicología Veterinaria

Analysis of seminal quality, a tool in fertility experimental toxicology study.

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Abstract

The methods of semen quality valuation in artificial insemination and research were suffering a constant development to estimate with more precision the males fertility. The objective of this work is making of a theoretical-practice guide that allows to carry out studies of the semen in rabbits, (New Zealand White breed), applied to the fertility toxicology thematic. Extensive results are obtained in our research in function of the female reproductive toxicity and the male in indirect form, this study allows to detect the absence or presence of reproductive toxicity directly in the male, to highlight that the results go or not more there is of the integrity of the sexual organs, anatomopathological exams, health, sexual libido, number of pregnant females, number of breedings and other variables. You should be formed 4 experimental groups from 8 to 10 rabbits males/group (32-40 total), of 7-8 months of age (adults) of 1,8-2,5 kg of corporal weight, forming a control vehicle group and three levels of the product to evaluate, being administered in repeated dose during 8 weeks (60 days for oral way, which embraces a spermatic cycle and ¼ of the following one, in the rabbit this cycle lasts 48 days), during this time it should weigh the animals weekly to determine the product doses to administer and they were observed twice a day. You can include or not a positive control group administered with reference substances, which can be administered by dose and different way to the assays substance. After 8 weeks it should extract the semen for their macroscopic and microscopic valuation with the use of an estrogenic female rabbit (1 female/6-8 male). For a bigger valuation of the toxicity product to evaluate in the male's reproductive system should carry out the anatomopathological study of organs and cavities, to extract (testicles, epididymis, prostate and seminal vesicles) for their histological study and the determination of the relationship of organ weight/corporal weight. All the analyzed variables should be compared against negative control group. This work is not based on the implementation of new techniques, but the researchers should knows that these tools exist and that they can be used to solve in an immediate way a problem given with methodologies that include a low cost, easy reproduction, quick obtaining of the results, they don't need of sophisticated teams, neither of qualified personnel in this stuff.

Keywords: Seminal quality, fertility, toxicology study.

Resumen

Análisis de la calidad seminal una herramienta en el estudio de toxicología experimental de la fertilidad.

Los métodos de valoración de la calidad del semen en la inseminación artificial e investigación están sufriendo un desarrollo constante para estimar con más precisión la fertilidad del macho. El objetivo de este trabajo es realizar una guía de la teórico-práctica que permite llevar a cabo estudios del semen en conejos, (Nueva Zelanda Blanco), aplicado a la temática de toxicología de la fertilidad. En nuestras investigaciones se obtienen extensos resultados en cuanto a la función de la toxicidad reproductiva en la hembra y en el macho de forma indirecta, este estudio permitirá descubrir la ausencia o presencia de toxicidad reproductiva directamente en el macho, para resaltar que los resultados van más hay de la integridad de los órganos sexuales, exámenes anatomopatológicos, salud, libido sexual, el número de hembras preñadas, el número de crías etc. Se deben formar 4 grupos experimentales de 8 a 10 conejos machos/grupo para un total de 32-40 animales, de 7-8 meses de edad (adultos) de 1,8-2,5 kg de peso corporal, formando un grupo control vehículo y tres niveles del producto a evaluar, administrándose en dosis repetida durante 8 semanas (60 días si es de forma oral para que incluya un ciclo espermático y ¼ del siguiente), en el conejo este ciclo dura 48 días. Durante este tiempo debe pesar los animales semanalmente para determinar la dosis del producto a administrar y debe observar los animales dos veces al día. Puede incluir o no un grupo control positivo administrado con sustancias de referencia, siendo posible la administración de esta en dosis y vía diferente a la sustancia de ensayo. Después de 8 semanas de administración debe extraer el semen para su valoración macroscópica y microscópica con el uso de una coneja hembra estrogenizada (1 hembra/6-8 machos). Para una mayor valoración de la toxicidad del producto a evaluar en el sistema reproductor del macho debe llevar a cabo el estudio anatomopatológico de órganos y cavidades, extrayendo los testículos, epidídimos, próstata y vesículas seminales, para su estudio histológico y la determinación de la relación peso del órgano/peso corporal. Todas las variables analizadas deben compararse contra el grupo control negativo. Este trabajo no esta basado en la aplicación de nuevas técnicas, pero los investigadores deben saber que estas herramientas existen y que pueden utilizarlas para resolver de una manera inmediata un problema dado con metodologías que incluyen un bajo costo, fácil reproducción, obtención rápida de los resultados, no necesitan de equipos sofisticados, ni de personal calificado en esta temática.

Palabras claves: Calidad seminal, fertilidad, estudio de toxicología.

Introduction

The methods of semen quality valuation in artificial insemination and research were suffering a constant development to estimate with more precision the males fertility.

One of the deficiencies found in the experimental toxicology studies is due to that in most of the validated assays and accepted that of course are the only ones that the researchers carry out, they try the toxicology fertility evaluation in the female.

It is analyzed the male in mating, measuring the sexual libido, number of pregnant females. In any case is made allusion to the spermatic concentration, volume, motility, spermatic morpho anomalies, which are decisive of a range of valuations to measure in these studies in a direct way, to mention some: the index of fertility, number breedings, viability of the breedings, malformations of the skeleton and of organs among others.

On the other hand it is used the rabbit like biomodel, since the same one is used as non rodent species in the studies of fertility and teratogenesis, besides overcoming in size to the rat, for that facilitates the whole process of extraction of the semen and appropriate quantity for everything in analysis that should be made.

The objective of this work is making of a theoretical-practice guide that allows to carry out studies of the analysis of seminal quality in rabbits, (New Zealand White breed), applied to the experimental fertility toxicology thematic.

This work is not based on the implementation of new techniques, but rather the researchers in the field of the experimental toxicology knows that these tools exist and that they can be used to solve in an immediate way a problem given with methodologies that include a low cost, easy reproduction and quick obtaining of the results, which don't need of sophisticate equipments neither of personnel qualified in this matter.

For that which we take valuations that include a basic analysis as they are the tests of routines that we debate in this work, being able to be developed by technicians or specialists in laboratories with low resources.

Content

Experimental Design

You should be formed 4 experimental groups from 8 to 10 rabbits males/group (32-40 total), of 7-8 months of age (adults) of 1,8-2,5 kg, forming a control vehicle group and three levels of the product to evaluate, being administered in repeated dose during 8 weeks (60 days for oral way, which embraces a spermatic cycle and ¼ of the following one, in the rabbit this cycle lasts 48 days), during this time it should weigh the animals weekly to determine the product doses to administer and they were observed twice a day. You can include or not a positive control group administered with reference substances, which can be administered by dose and different way to the assays substance. After 8 weeks it should extract the semen for their macroscopic and microscopic valuation with the use of an estrogenic female rabbit (1 female/6-8 male). For a bigger valuation of the toxicity product to evaluate in the male's reproductive system should carry out the anatomopathological study of organs and cavities, to extract (testicles, epididymis, prostate and seminal vesicles) for their histological study and the determination of the relationship of organ weight/corporal weight. All the analyzed variables should be compared against negative control group.

Semen Obtaining

Induction of the female's zeal:

It should put hormonal treatment with PMSG (chorionic gonadotropin of the pregnant mare serum). Example of some effective application schemes:[1]

*12-35 I.U, I.M or S.C way, 48 h before the moment of the natural service, the dose 35 I.U are more effective.

*20 I.U, I.M way, 72 h before the moment of the natural service.[2]

To improve the acceptance should be bio-stimulator to males with a treatment of 16 h of light during 8 days before the semen extraction.[1,2]

Semen extraction method:

The female is taken to the male's cage. The female is located in position of service, when the male attempts the jump the rustic or factory artificial vagina it is placed of below the belly of the female rabbit, in such way that the penis of the reproducer is introduced in the artificial vagina.[3] The temperature of the water of the artificial vagina should be such that arrives to the penis from the male to 40 / 42°C. It is observed in the collector tube if the one ejaculated presents mucous plug or gel, coming from the secretion of the seminal vesicles and the prostate, should move away. Then it is located in a recipient in water bath at 37°C to avoid thermal crashes.[4]

Macroscopic Test [5]

- COLOR: White pearly (good), another color is classified as bad.
- ASPECT: It is more wanted uniform aspect and don't uniform aspects as for the opacity.
- SMELL: *Suis generis*, another smell variant is classified as bad.
- PH: (6,8-7,3) is normal. Different value to these indicate a bad seminal quality (to determine with pH-meter or sunflower paper).
- VOLUME: It is determined by means of the tubes collectors graduation, it should be of 0,25-1 mL (autumn) to 0,3 -1,5 mL (spring).
- CONSISTENCY: The best are liquid. In first instance homogenize the sample of semen slowly, then takes 20 µL and it allows to fall on a slides sheet drop to drop, classifications: Drop to drop, filament form or it flows as the water.

Microscopic Test [3-6]

The microscopic test to carry out by two independent observers and to settle down average between both observers.

- *CONCENTRATION:*

Technique 1: To carry out a 1:100 dilution in saline solution with formalin, homogenize the sample, take 10 µL and let slip in the Neubauer chamber. Stand for 2 min and then perform the count.

Saline solution with formalin: 1 L final volume (9 g NaCl +3 mL formalin 40% + 1000 mL of distilled water). To determine the sperm present in the 4 quadrants, add and multiply x 4 and then by dilution (100) and for the mounted volume (10).[6,7] Concentration = 50 x 4000 = 0,200 × 10^6 sperm/mm^3. You can also calculate the total sperm per ejaculate, it is necessary to unify the measure we should be multiplied again by 1 000 to take from mm^3 of concentration up mL volume. Example: ejaculate volume: 1 mL, concentration: 0,200 × 10^6 sperm/mm^3 (1 x 0,200 x 10^6 x 1000 = 0,200 x 10^9 total sperms).[7,8]

Technique 2: Homogenize the sample in assay tube add 100 µL of semen + 900 µL of diluent solution, or a 1:100 dilution, homogenize the sample slowly and it mount in the Neubauer chamber, let settle for 5 min. You must have the central mm of the chamber. The sperm counted multiply by 4 or 5 quadrants and divided by 10, it reports the number of 10^6 sperm/mL.

Preparation of the diluent solution: 50 g of Sodium Bicarbonate+distilled water until completing to 100 ml of final volume.[8-10]

- *ANOMALIES:*

Can be colored or not the sample, you should express the results in (%). You should placed a drop in a slide and it is observed:[10]

1.The head, normal and abnormal (macrohead, microhead, double and it looses).

2.In the neck, normal and abnormal (coiled, double, thickened, and eccentric).

3.In the tail, normal and abnormal (coiled, whip, double and bent).

Of being colored the sample to take 0,1 mL of semen+3 mL of NaCl 0,9%, homogenized the sample and it added from 3 to 7 drops of eosin Y 1%, 5 min to stand and to place a drop between slide and coverslip. We will accept 10 to 15% of anomalies or 20-30% according to other authors.[11]

- *MOTILITY:*

Two types of movements: rotation (about its axis) and progressive movements (displacement of the cell, is the most important). All material to be used at 37°C.[9-11]

Masal Motility: Are deposited within 7 to 10 mL of this one slide, was observed at 40 magnification. You should appreciate the speed with which the whirls are moving from the surface of the drop (score scale 0-5). None (0), trembling in the place (1), beginning of waves come and go, is still bad (2), regular slow waves (3), good fast wave (4) and finally very good with whirls or tornadoes (5). The tested animals must be between 4 and 5 (60-70% is good), it is essential to establish an average between the number of animals per dose.[11,12]

Individual Motility: Semen must be diluted (100 µL of semen for 900 µL of NaCl 0,9% at 37 º C), homogenize the sample and then place a drop between slide and coverslip, observed under light microscopy. It evaluates the % of sperm motility regardless of their movement and then must evaluate the type of movement (progressive, rectilinear and uniform) on a scale of 1 to 5, Very Good (5= >95%), Good (4=80-95%), Regular (3=70-80%), Bad (2=60-70%), None or very poor (1= <60%).[12,13]

- *VIABILITY:*

Alive/dead value. Must be measured within 30 min after the extraction. 5 drops are added to 1% nigrosine homogenate remaining in the test for determining anomalies, homogenize the sample and wait 5 min, the eosin stains dead sperm pink and nigrosine allows live (colorless or transparent) are displayed, then homogenized and performed the smear of a drop, drying for 10 min. More than 70-80% (very good), 70% (good), 60 - 69% (regular), and below 60% (poor).[14,15]

Organs weigh and anatomopathological exam

To exam the content of the abdominal, thoracic and cranial cavities, to weigh (right and left testicles, right and left epididymis, prostate and seminal vesicles) and to take samples for the histopathological study (to fix in formaldehyde 10% buffered and to color with haematoxylin and eosin).[16] Calculate the relationship: (organ weigh/corporal weigh) x 100, in the sacrifice day. To analyze with priority the animals of the control group and the maximum dose used.[17]

Statistical analysis

All the analyzed variables should be compared against negative control group. The continuous variables were analyzed with a variance analysis test (ANOVA) if it completes such suppositions and those categorical by means of the homogeneity **χ^2** no parametric test. The level of established significance should be of α=0,05.

Conclusions

This work is not based on the implementation of new techniques, but is very important that the researchers that work in the experimental toxicology thematic knows these tools and that they can be used to solve in an immediate way a problem given with methodologies that include a low cost, easy reproduction, quick obtaining of the results, they don't need of sophisticated teams, neither of qualified personnel in this stuff. The use of these tests in the experimental toxicology laboratories constitutes a useful tool, which helps to a wider new products preclinical evaluation and active ingredient.

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