Potency and Purity of Equine Chorionic Gonadotrophin

Equine Chorionic Gonadotrophin (eCG) is a biological extract. It is produced by the endometrial cups of the chorionic girdle of the mare's placenta, from day 40 to 130 of pregnancy. In the mare, eCG has a luteinising hormone (LH) effect, resulting in the luteinisation of follicles to create secondary corpora lutea (CL) on the ovaries. These secondary CLs produce progesterone to assist the primary CL in maintaining pregnancy until the placenta takes over all progesterone production, around day 150 of pregnancy.

eCG is collected from the serum of pregnant mares, hence its previous name, pregnant mare serum gonadotrophin (PMSG). Following collection, it is purified, sterilised and freeze-dried for use in reproduction programs in cattle and other species. In cattle, eCG has dual action, binding to both FSH and LH receptors. It is widely used to increase pregnancy rates in synchrony programs for anoestrous cows.

Testing eCG potency

The potency of eCG can be tested using either biological or enzyme-linked immunosorbent assay (ELISA) methods.

The biological method is the pharmacopoeial method, as described in the European Pharmacopoeia (EP). This test measures the effect of eCG on increasing the mass of ovaries of prepubertal female rats. The test product is compared with a reference preparation or international standard, calibrated in international units.

The biological method remains the only internationally approved method for testing products containing eCG prior to release for use in animals. At least 30 similar (age and weight) rats are required per batch of product tested. Various dilutions of product are tested and the ovarian response quantified against standardised materials. Injections of the test solutions are administered at six time points to each of the lab animals. This test has relatively wide confidence limits due to its biological nature.

An ELISA test has been developed to reduce the dependence on tests involving lab animals. This test is solid phase and is based on the 'sandwich' principle.

The microtitre wells are coated with a monoclonal antibody directed towards a unique antigenic site of the eCG molecule. An aliquot of sample containing endogenous eCG is incubated in the coated well. After washing, a second incubation follows with enzyme conjugate, which is an anti-eCG antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off. The amount of bound peroxidase is proportional to the concentration of eCG in the sample. After adding a substrate solution, the intensity of colour developed is proportional to the concentration of eCG in the sample.

The ELISA method produces a test result more quickly than the biological assay, and is not susceptible to biological variation. Careful handling of the materials is still required, but the ELISA test generally has a tighter confidence limit. Regardless, the ELISA test is not an approved method for testing veterinary medicine products for release to market.

eCG products from around the world

Several eCG products were tested in 2014 for their potency using the biological test. The results are illustrated in figure 1.

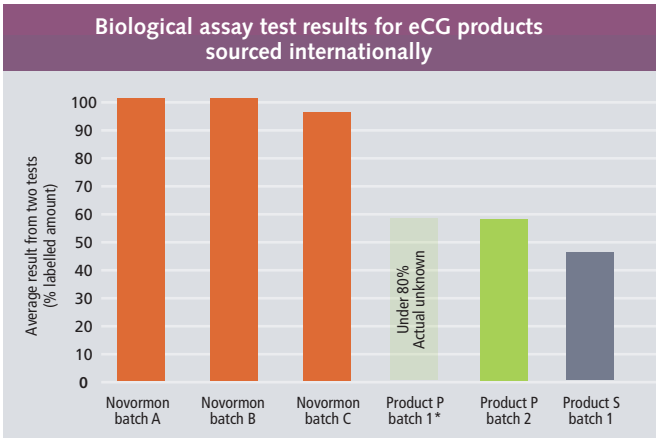

Figure 1. Results from biological assay testing of products sourced from Argentina, New Zealand and Brazil.

**no ovarian response detected as serial dilutions were made expecting a minimum result of 80% of label claim*

Pre-testing via ELISA method was required for Product P batch 2 and Product S batch 1 to determine appropriate dilutions to test using the biological assay.

Secondary testing using the ELISA method was required to determine suitable dilution factors for the biological assay as several samples did not meet the minimum standard for the biological test (80% of label claim). ELISA test results can be seen below.

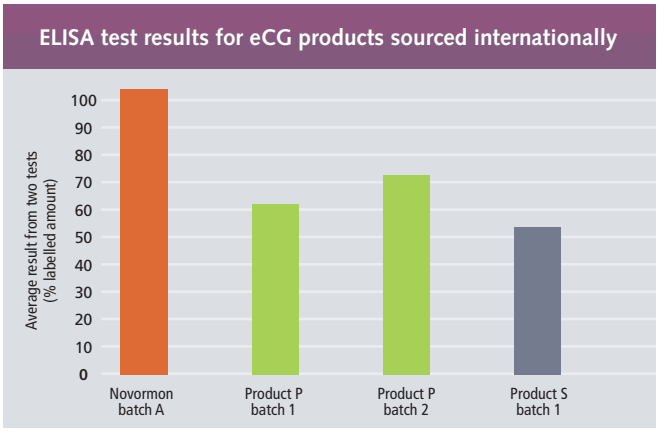

Figure 2. Results from ELISA testing of products sourced from Argentina, New Zealand and Brazil.

Discussion

No ovarian response in prepubertal rats could be detected with the first batch of product P that was tested. The protocol for this biological assay, as directed in the European Pharmacopoeia, involves serial dilutions in three groups of prepubertal female rats. The serial dilutions were made expecting the potency to be 80 - 125% of the labelled amount. As the potency was outside of this range, the biological assay could not be specifically quantified (but was below 80% of stated label claim).

To overcome this problem, the ELISA test was performed on subsequent eCG batches, including product P batch 2 and product S prior to performing the biological assay. The ELISA test result provided an estimate of the likely eCG potency, meaning that dilutions of the product were made corresponding to likely potency. This additional testing allowed quantification of the biological potency of the various eCG products via the approved testing method.

Conclusion

Every batch of eCG produced for use as a veterinary medicine must have its potency tested and meet the stringent criteria set in the European pharmaocopoeia. Determining the biological activity, as described in the EP, remains the only approved method for eCG testing.

The testing performed on various eCG products showed that certain eCG products on the market had lower potency than the label states. These lower than expected test results were identified by both biological and ELISA test methods.

Novormon eCG available in the NZ market consistently meets all label specifications, including potency.

Reference

European Pharmacopoeia 6.0. Gonadotrophin, equine serum, for veterinary use. 01/2008:0719

Addendum

Further testing of eCG products sourced from the NZ market was undertaken in 2015. Results of the biological and ELISA assays were consistent with those reported in 2014.

To verify the results, samples of both eCG products were then acquired in NZ and sent together to an independent laboratory in Europe. This organisation is at the forefront of eCG laboratory testing and verification of international products[1]. The results from this testing are shown in figure 3.

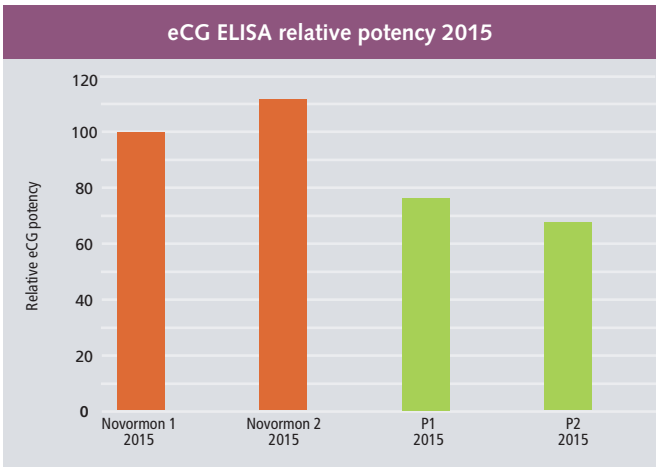

Figure 3. Relative eCG potency of products sent from NZ in August 2015

It was noted by both laboratories that one of the batches of product P had undissolved (insoluble) particles following reconstitution. As eCG is quite soluble, this was determined to likely be contaminant proteins rather than eCG. The result reported was determined from the supernatant (portion that was in solution).

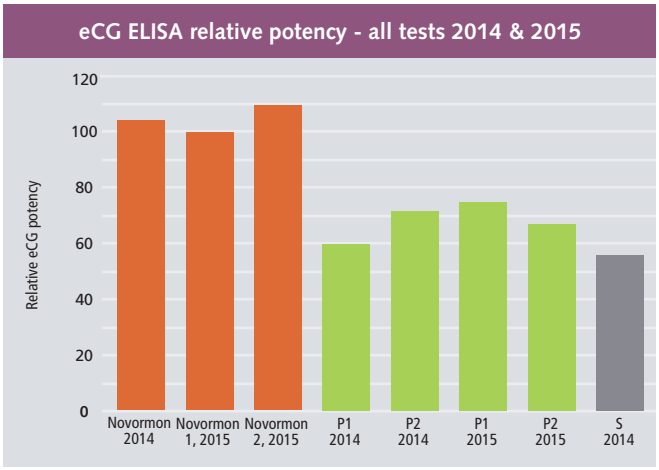

Figure 4. Relative eCG potency from ELISA testing of various products over two years

In summary, the combined results from testing in 2014 and 2015 are shown in figure 4. Novormon remains the eCG of highest quality and potency available on the New Zealand market.

[1]Lecompte, F. Roy, F and Combarnous, Y. 1998. Journal of Reproduction and Fertility 113, 145 - 150