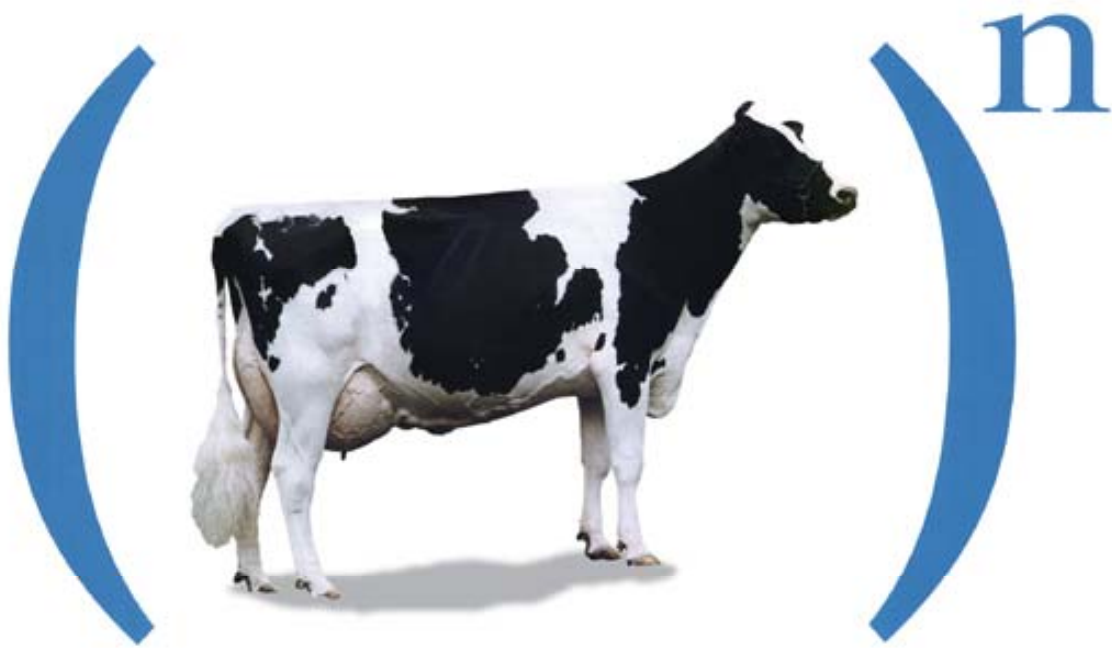




LABORATORIOS CALIER, S.A.

# FREQUENT ASKED QUESTIONS



**pluset<sup>®</sup>**

Superovulation raised to maximum potency



## FAQ's OF PLUSET®

- **What is Pluset®?**

Pluset® is a porcine pituitary extract (PPE), which contains both of the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). This product is primarily used to induce development of ovarian follicles during the superovulatory process in ruminant females. The objective of superovulation is to induce multiple follicles to grow and ovulate so that their oocytes (eggs) can become fertilised and the resulting embryos can be collected from genetically valuable donor females and subsequently transferred to recipient females that will gestate the embryos to term.

Because of its stimulatory activity on follicular growth, Pluset® is also used in cases of reduced fertility (heat stress, high milk production, ovarian inactivity, etc...) to supplement endogenous FSH synthesis and release from the pituitary.

- **How is Pluset® produced?**

The production process comprises a number of extractions with solvents and fractionated precipitation, in processes that take 15 days. Independent PPE fractions are produced, one for FSH and one for LH from porcine pituitary glands from certified healthy pigs. In addition, the extraction method is validated against any viral contamination. To ensure the quality of the final product, the FSH fraction and the LH fraction are each independently analysed for both FSH & LH bioactivity. The final product is a mixture of both fractions, in a proportion to ensure a FSH:LH ratio of 1:1 in terms of biological activity, expressed in International Units (IU).

- **Why does Pluset® contain LH?**

LH, or luteinizing hormone, acts synergistically with FSH to facilitate ovarian follicular growth and maturation. Characteristic of early follicular phase growth, ovarian follicles less than 9 mm in diameter are FSH-dependent, evidenced by the presence of FSH receptors in the granulosa cells, but at this stage, there is a minimal presence of LH receptors. When the growing follicles reach 9 mm in diameter, the FSH receptor disappears from the granulosa cells while the LH receptor is more



abundant, essentially causing a switch in the follicle's dependence from FSH to LH. Thus, the LH hormone is necessary for follicular growth in the final stages.

Previous research has shown that gonadotropin preparations containing FSH suppress endogenous LH release, to the point of inhibiting final growth and maturation of the follicles and can even prohibit ovulation. Stress during the treatment period can also induce cortisol release, which has a direct negative impact on LH release, thus exacerbating endogenous LH suppression. The LH portion of Pluset<sup>®</sup>, therefore, can supplant this hormone, when endogenous LH is suppressed, insuring that the superstimulated follicles have the opportunity to complete their growth and maturation.

- **Why are FSH and LH in a 1:1 ratio?**

The reason for a 1:1 ratio of biological activity in Pluset<sup>®</sup> is because of its historical predecessor, Pergovet<sup>®</sup>. Pergovet<sup>®</sup>, was human menopausal gonadotropin, or hMG, that was used because of its excellent results and homogeneity, when compared to other, then existent, FSH compounds. Pergovet<sup>®</sup> was a very expensive drug to manufacture, and thus, a decision was made to develop a PPE with the same characteristics as hMG, namely Pluset<sup>®</sup> with its 1:1 FSH:LH ratio in biological activity.

- **Does a 1:1 FSH:LH ratio mean the same amount of each hormone?**

No, not in terms of physical weight or amount. It simply means that there is the same number of International Units (IU's) of each hormone and the number of IU's is based on biological activity. However, since the FSH and LH hormones have a different molecular structure, they are measured by different methods of analysis (i.e. *an IU of FSH is not the same as an IU of LH*).

The quantity of each hormone is therefore, equivalent in terms of bioactivity and proportion of its standard. It also must be taken into account, however, that the half-life of LH in circulation in the cow is much shorter than FSH.

The biological activity per mg of the LH-fraction of Pluset<sup>®</sup> is much higher than that of FSH, but its half-life in circulation is about 25 minutes for LH compared to about 2.5 hours for FSH. The FSH-fraction of Pluset<sup>®</sup> contains some 25 IU/mg, whereas the LH fraction contains some 300 IU/mg. This means that, in each 500 IU vial, there is approximately 20 mg of FSH and 1.5 mg of LH (i.e. mg of WEIGHT, *not* mg of NIH-



FSH-P1 standard). Pluset® cannot, therefore, be compared directly with products that use NIH mg standards because of their different methods of analyses. Pluset® uses a standard measuring biological activity, whereas NIH mg standards measure by radio-immunoassay (RIA), which is an antibody-based standard that does not measure bioactivity.

- **Why is Pluset® measured in units of biological activity?**

Extractions from biological substrates give different performances, depending on the original raw material, which will present slight variations (depending on the age and gender of the animal, season, etc.). Some gonadotropin products express hormonal content in mg per unit of volume, which is measured by radio-immunoassay (RIA), an analytical technique that employs antibodies to FSH and LH. These hormones, however, as well as their metabolites, may have several isoforms, some of which are biologically active while others are inactive. It is also known that other hormones such as hCG or PMSG, may show a high degree of FSH or LH activity by bio-assay, which will not be indicated by RIA, due to the lack of recognition of these hormones by FSH or LH anti-bodies.

Therefore, RIA's for FSH could falsely measure inactive forms as active, and inversely, fail to measure active forms at all (false positives and negatives).

Our biological analyses measure only these important bio-reactive molecules, which express FSH and LH activity. In this way, each vial always contains the same amount of International Units and the same FSH:LH ratio. Thus, the product is always the same, and the biological response is more predictable.

- **How is biological activity measured?**

The methods of analysis employed are the Steelman-Pohley method for FSH, and the Parlow method for LH.

For the assays, prepuberal rats are injected with the compound. To determine FSH, the increase in ovarian weight is measured after 4 days. To determine LH, depletion of ascorbic acid in the ovary is measured after 4 hours.



- **Why are these methods of analysis used?**

These methods are the only ones recognised by the World Health Organisation (WHO) and *European Pharmacopoeia* to measure biological activity of these two hormones, as they are the most reliable and specific. They each measure the activity of the hormone, even in the presence of high concentrations of other compounds, thereby not interfering with the results.

The Steelman-Pohley method for FSH measures *ovarian growth*, which is the goal of the superovulation. At a minimum, three replicates of each sample are conducted, in order to ensure statistical significance of the results and to develop a parallel-curve, as described in *European Pharmacopoeia*.

- **What dosages should I use?**

Holstein dairy cows:	800-1000 IU
Beef European cows:	600-800 IU
Heifers (dairy, beef):	500 IU
Zebu:	250 IU
Sheep, goats:	250-350 IU

Typically, declining doses of the above total dosages are administered over a 4 day period at 12 hour intervals, in addition to a prostaglandin-F<sub>2α</sub> (PGF<sub>2α</sub>) on the 3<sup>rd</sup> or 4<sup>th</sup> day.

Recommended schedule for 800 IU in 4 days:

Day 1	08:00 hrs	3.0 ml i.m.	(150 IU FSH + 150 IU LH)
	20:00 hrs	3.0 ml i.m.	(150 IU FSH + 150 IU LH)
Day 2	08:00 hrs	2.5 ml i.m.	(125 IU FSH + 125 IU LH)
	20:00 hrs	2.5 ml i.m.	(125 IU FSH + 125 IU LH)
Day 3	08:00 hrs	1.5 ml i.m.	(75 IU FSH + 75 IU LH)
	20:00 hrs	1.5 ml i.m.	(75 IU FSH + 75 IU LH)
Day 4	08:00 hrs	1.0 ml i.m.	(50 IU FSH + 50 IU LH)
	20:00 hrs	1.0 ml i.m.	(50 IU FSH + 50 IU LH)



Recommended schedule for heifers (500 IU):

Day 1	08:00 hrs	2.0 ml i.m.	(100 IU FSH + 150 IU LH)
	20:00 hrs	2.0 ml i.m.	(100 IU FSH + 150 IU LH)
Day 2	08:00 hrs	1.5 ml i.m.	(75 IU FSH + 75 IU LH)
	20:00 hrs	1.5 ml i.m.	(75 IU FSH + 75 IU LH)
Day 3	08:00 hrs	1.0 ml i.m.	(50 IU FSH + 50 IU LH)
	20:00 hrs	1.0 ml i.m.	(50 IU FSH + 50 IU LH)
Day 4	08:00 hrs	0.5 ml i.m.	(25 IU FSH + 25 IU LH)
	20:00 hrs	0.5 ml i.m.	(25 IU FSH + 25 IU LH)

- **How many animals can I treat with a package of Pluset?**

1 dairy cow                      2 heifers  
 1.4 beef cows                  4 zebus  
 3-4 sheep or goats

Since there is more biological activity per unit packaged, more animals can be treated than with other commercially available products.

- **Is it important to adjust the dose?**

The cow is a monovulatory specie, thus, it typically ovulates a single ovum, and in some instances, two. This implies natural mechanisms of self-regulation through the dominant ovarian follicle, which normally inhibits the development of other follicles. When administering gonadotropins, the induced growth of multiple dominant follicles can significantly alter fertility. Growing follicles produce estrogens, and can be produced in excess, which can cause oedema in the oviduct, which can in turn inhibit normal fertilisation of the oocytes.

Over-stimulation, or excessive ovarian development, is usually accompanied with poor results, typically characterised by large numbers of non-fertilised or degenerative ova. To avoid this, the dose should be reduced to a dose that yields acceptable percentages of normally developed embryos. Consider that would be preferable to under-dose than over-dose.



- Which other uses does Pluset® have?

There are some cases of low fertility due to a deficit in endogenous production of FSH. This can happen with stressed animals (e.g. - heat-stress), poor or insufficient feeding, or high milk production. In these cases, silent heats may occur and ovulation may not be occurring.

The administration of 200 IU of Pluset® in a single i.m dose may help to re-establish ovarian follicular development and ovarian cyclicity. If the presence of a *corpus luteum* is detected, PGF<sub>2α</sub> could be administered in 3-4 days to induce oestrus and eventual ovulation of the developed follicle.

*For any additional information, do not hesitate to contact us at:*

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