C6: a small molecule that triggers ovulation

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Abstract:

Currently used methods to trigger ovulation in livestock (PSMG, mainly) are not satisfying in terms of ethics, and safety. This paper provides new insights to control the reproductive function in livestock, by mimicking the natural process leading to ovulation using a chemical compound called C6. C6 is a candidate to replace the kisspeptin, which is a natural molecule that controls the secretion of GnRH by the hypothalamus. This paper analyzes the effects of C6, and clarifies the biological pathways used by the molecule leading to ovulation. C6 is promising and may be usable as a veterinary drug, without the drawbacks of PSMG treatment.

Keywords: Drugs ; GnRH ; Kisspeptin ; Livestock ; Ovulation ; Reproduction ; Reproductive control ;

I Introduction

The reproduction of small ruminants is seasonal. In our latitudes, animals are sexually active from the end of summer to the end of winter. The result is a marked annual fluctuation in the production and the prices of dairy product and meat. In general, the methods of reproductive control in these species aim on the on hand to better synchronize ovulation and, on the other hand, to restart birth during the year. Reproductive control methods are mainly based on the use of hormonal treatments. These treatments are effective but generate side effects, both for the animal and for the environment.

In the year 2000 researchers discovered a small molecule called Kisspeptin (Kp). This molecule has the capacity to function at the neural level and is capable to trigger a series of events within mammals allowing to induce ovulation. In scientific literature, we noticed that among females hit by pathologies leading to sterility, among mammals in a period of non-breeding seasons the rate of some hormones like LH (Luteinizing Hormones) was extremely low. This hormone allows ovulation to start. The administration repeated Kisspeptin, seeing the infusion on 48h allowed the ovulation to start (figure 4C).

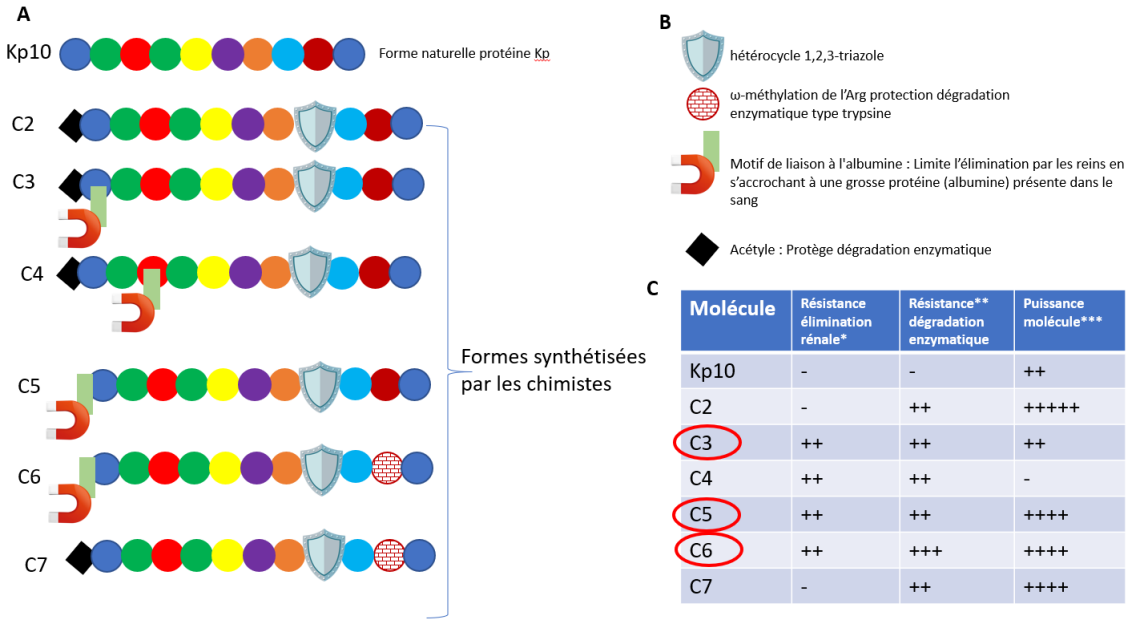
The idea to administer Kisspeptin to sheeps to trigger ovulation was not conceivable in a practical point of view. It is hard to imagine a breeder performing an infusion during 48 hours to get the expected results. The problem is not in the quality and the power of the molecule but its life expectancy. In fact, the Kisspeptin is very quickly degraded by the body at two levels. The first level of degradation is done by enzymes at strategic points of the molecule.

The second is the elimination concerning the kidneys, this molecule is very small. At this point, the Kisspeptin has a lifetime of a few minutes. The aim of the researchers was to create a molecule very similar to the Kisspeptin with the same function. This molecule would have a resistance to the degradation and could stand for a few hours. This modification and its level of action are presented in this publication.

II First stage: modified the Kisspeptin to make it resistant to degradation.

In essence, we can compare the Kisspeptin to a key and its receiver KISS1R to a lock. This interaction Kisspeptin/Kiss1R will activate the neuron and will create a chain of actions that will activate ovulation. First, the chemists tried to modify the structure of Kisspeptin to make it more resistant over time. However, changing its structure equals to changing the form of the key.

That's why chemists have tried different modifications (Figure 1). All the molecules have been tested on cells possessing kiss1R. Thanks to very specific reagent, the biologists were able to see the liberation of intracellular calcium and can determine with precision if the interaction kiss/KISS1R (or Key/lock) is effective.



C

Molécule	Résistance élimination rénale*	Résistance** dégradation enzymatique	Puissance molécule***
Kp10	-	-	++
C2	-	++	+++++
C3	++	++	++
C4	++	++	-
C5	++	++	++++
C6	++	+++	++++
C7	-	++	++++

Figure 1: A : Natural forms and set of synthesized biochemical modifications. Each circle of color represents an amino acid. The forms represented in B which correspond to the role of the modification carried out by the chemists. C : Board representing the set of results obtained after measurement of the interaction Kisspeptin/KISS1R
Non tested in the laboratory but based on their bibliographical knowledge base and of tests in vivo realized previously (like C2). tested with a blood serum*
The interaction behind the Kp and its receiver causes a chain reaction in the cell that releases Ca2+ (which is measurable). The more the molecule is powerful, the more the reaction will be made with a small quantity of molecules

With all these results, the C3, C5 et C6 composites have become very good candidates. Their efficiency but also their resistance to degradation were better than Kisspeptin. We need to know that, before obtaining different composites, a hundred antagonists were tested (see previous publications).

III Second step: test on the sheep to verify its ability to induce ovulation

For this step, several experiences were necessary. At first, the biologists sought which of the three compounds was the best. They carried out regular blood tests, for eighteen hours. Measuring the hormone that causes ovulation in females called Luteinizing hormone allows researchers to see the effectiveness of different compounds (Figure 2).

Once the best candidate was selected, the researchers tested different quantities of molecules to inject: between 1 and 45nMol. They realized a new test of blood to make sure to measure the LH and then estimated what the best dose was. Then the researchers realized this work in period of seasons and the off-seasons with new measures of hormone like progesterone. It allowed to confirm if there is or is not a triggering of ovulation.

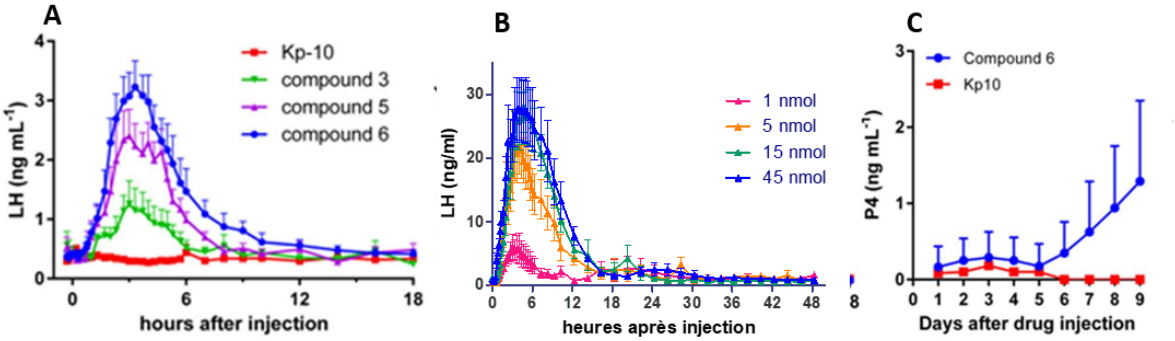


Figure 2: A: Concentration of LH measured in the ewes in the course of time after the injection of the different compounds and natural molecule Kp as control. B: Concentration of LH measured in the ewes in the course of time after the injection of the different quantities of the compound C6 C: Progesterone concentration measured in ewes in the time of the off-season period (Decourt et al., 2016)

When one of the compounds is injected at once, we can see that this triggered the release of LH in the sheep while the natural form can't do that. The best candidate seems to be the compound C6. The more important the quantity of C6 is, the stronger the concentration of LH. It also lasts longer. Nevertheless, we can see between 15 and 45 nMol, the difference of concentration of LH measured isn't interesting. The best dose retained by the researchers is 15nMol. This quantity injected is extremely weak: 15 nMol of C6 corresponds to the weight of half a grain of sand.

IV Third step: concretize the results

Having responses to prove that there is ovulation is good but… Seeing a lamb born thanks to the injection of C6 is better! To do this, the researchers carried out an injection of C6 to a dozen ewes. They then brought a ram to each of them. Their behavior was observed, and the females showed typical behavioral signs of a sheep in heat (Figure 3).

They then left rams fitted with marking straps with the ewes overnight. The next day, all the ewes had a mark suggesting that mating had taken place. Five months later, 7 out of 10 had babies which were all in good health. These results are similar to those obtained with conventional treatment.

Figure 3: Mating of a ram with a marking strap: when mating is attempted, the ram leaves a mark on the ewe's back

V Fourth step: Confirm that the molecule passes through the expected biologic mechanisms:

It takes more than cell testing to prove that. Indeed, an organization is much more

complex. This is why other teams have shown that C6 acts like natural Kisspeptin. By various means:

Verification of neuron stimulation at GnRH

First, we need to find and isolate GnRH neurons. How to find some very specific neuron through hundreds of millions?

Some culture to understand: GFP (Green Fluorescent Protein) was described the first time in 1962. Discovery by the Japanese Osamu Shimamura in a special kind of jelly fish Aequorea Victoria, she possesses like its name indicates the amazing feature of being a fluorescent protein. During 30 years, this protein remained a simple curiosity, we don't even know what its fluorescence can bring to jellyfish. And yet from 1992, the GFP has become in a few years one of the most powerful tools in molecular and cellular biology. The cellular biology takes over the GFP in 1992, the American Douglas Prasher had a brilliant idea: use the GFP to follow the expression of the genes. The idea is to attach the gene of the GFP just next to the gene you want follow: if in a given cell the gene that we want to follow is expressed, the GFP will be too. We can then identify it thanks to its fluorescence, with the help of a microscope.

(Explanations: David Louapre « Amazing science »)

In this research, researchers used several transgenic mice GnRH-GP to situate the neurons which make fluorescence. This way, they separate like the 29 neurons of GnRH (which have the KISS1R) and they are known to cause the ovulation. Researchers were able to measure their electric activity. The neurons activity of GnRH increases in the accordance with the concentration of C6 which confirm the possibility that the molecule takes place on the KISS1R present in the cellular membrane of the neuron of GnRH (Figure 4).

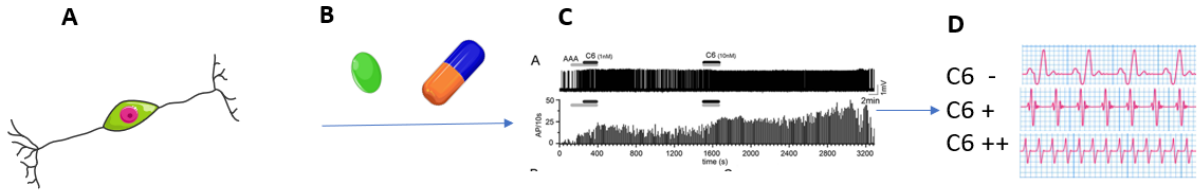


Figure 4: Schematic experimental measurements of the activity of GnRH neurons, known for their role in the triggering of ovulation, and carrying KISS1R. A: 29 neurons were isolated, and drugs have been added to prevent cross reactions. C: Thanks to measurement tools, researchers can quantify the electrical activity of a neuron. The higher the frequency, the more the neuron is active. D: Simplified representation of the results of experiments using C6: Activity of the nerve cell without C6, C6+/++ -> Activity of the nerve cell with a low or high C6 concentration.

The activity of GnRH neurons seems to be increased as a function of the concentration of C6, which supports the hypothesis that the molecule binds to the KISS1R present in the cell membrane of the GnRH neuron (Figure 4).

The principle of the key and lock tested

The mice KO/KISS1R are transgenic mice that don't have the KISS1R receptor. The

principle is to verify that by injecting the C6 (new key), the concentration of LH will not increase. If it doesn't increase, it means there is not the adequate lock. Since the researchers removed only one lock (the KISS1R) it'll prove that the C6 is specific to the Kiss1R. For this the researchers have injected some mice KO of the C6 then they measured the LH concentration.

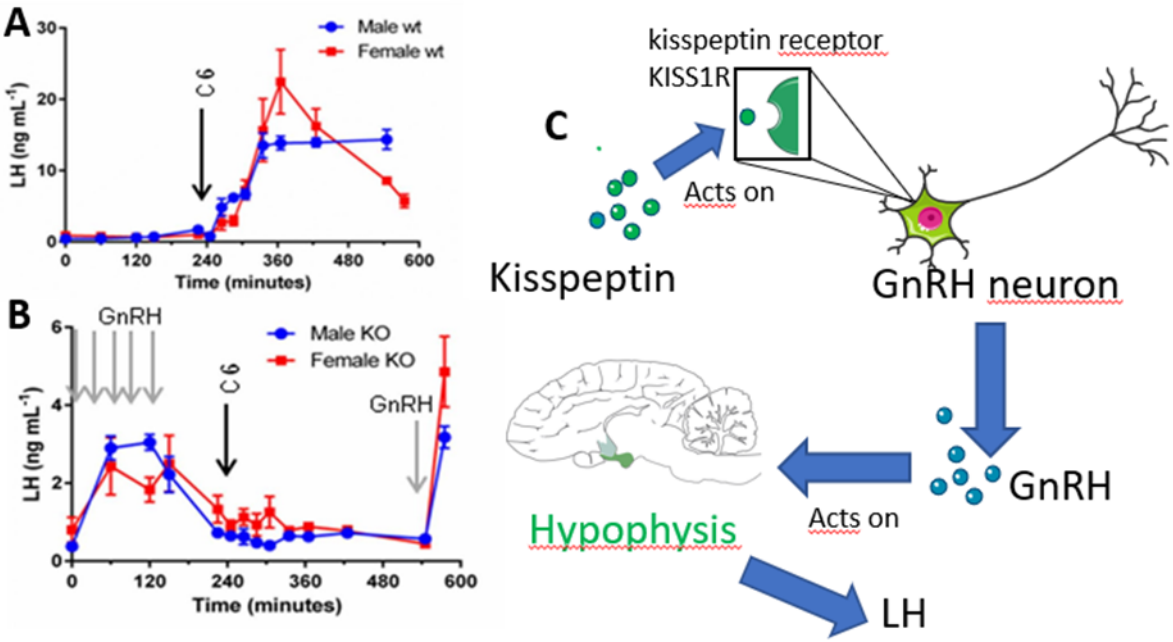


Figure 5: LH Dosing in control mice. B: LH dosing in KO-Kiss1R mice. We can observe that the injection of GnRH in the KO mice. The objective of the injection was to prove that the system works properly for KO mice. By injecting GnRH, we restore the LH production, proving that the missing Kiss1R in KO mice is concerned. C : Reminder of the action mechanism of Kp or C6 on the LH production (Decourt et al., 2016).

C6 increases the plasma concentration of LH in male and female mice but has no affects. On the secretion of LH in Ko Kiss1R mice, this data shows the C6 is everything (Figure 5A and 5B).

VI Conclusion

C6 is a synthetic compound that looks like Kisspeptin (a natural molecule that triggers ovulation) but has the great advantage of resisting enzymatic degradation as well as degradation by elimination through the kidney.

C6 will interact with the Kiss1R receptor present in neurons at GnRH. Its neurons will then activate and produce GnRH.

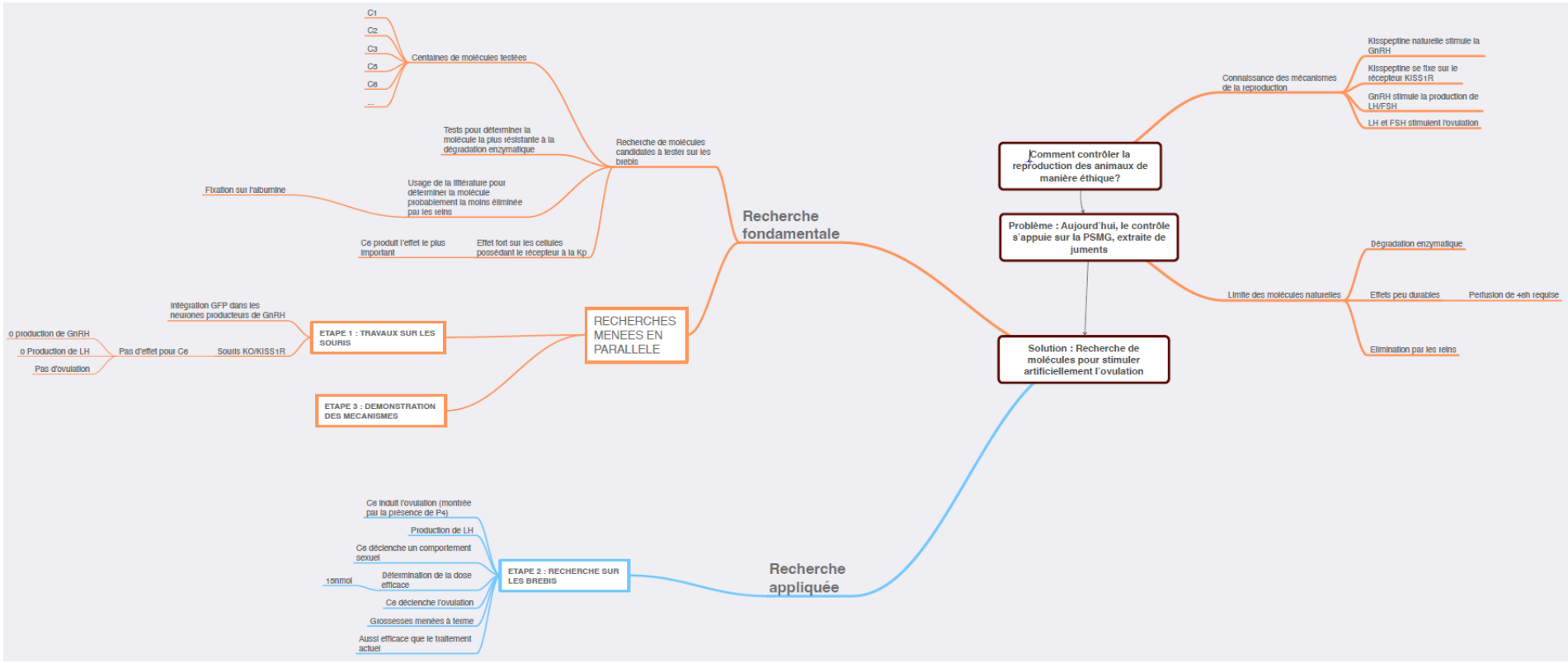
When the C6 is injected in the muscle of the sheep, this one will go through the animal by blood circulation and trigger the neurons at GnRH. Then a series of interactions already known will trigger the ovulation.

The animal will become in heat. The sheep will in the case of fertility, will become pregnant. During the tests, all the sheep from this technique were in good health. The results of fertility were the same as normal cases.

The C6 can become veterinary drugs and can be another choice instead of the other methods currently criticized.

However, the number of tested animals is not sufficient to pass this molecule into veterinary medicine. It would be necessary to try on a most consequent number and on other races of ewes. In addition, studies show that there may have other trails of treatments at the PMSG: Male effect, synthetic PMSG, etc.

C6 could also help in procreation of animals endangered with reproductive difficulties. In fact, the high stress of some animals in zoos reduces their fertility. The research team has set up collaborations with teams working on this subject, but this is another his…publication.



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Ont participé au travail d'écriture de cet article, en collaboration avec Vincent Robert, technicien de recherche en physiologie (par ordre alphabétique) : AYOUB S., BOUKROUNE Inès, CHAMPAGNE—CABLEY Maya, DECAMPS Léo, DELCAMPE Laurie-Anne, DELETTRE Louise, DENORME Vivien, FAY Marie-Pauline, GREUET Jade, GUYOT Léon, HARTL-HAZETTE Armand, LASSELIN Lou-Anne, LELENTA Barou, LEMAIRE Maxime, LESQUELIN Maxence, NAUDIN Laura, PAVIE-LEGRAND Léa, POREAUX Angeline, POULLAIN Loona, PREIRA Maryam, PREVOST Hugo, SOLARI Charlotte, TAQUET Avrile, THIEBAUT Quentin, THOMAS Charlotte, ZGODA Antoine.

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