Effect of the Reduction in the Frequency of Injections of Pituitary Extracts During a Superovulation on the Embryo Collection Results in Cows in Algeria

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Abstract

The aim of this study is to verify the efficacy of a superovulation treatment with 3 injections of pituitary extracts (Stimufol®) 24 hours apart and compare the results to a standard protocol (8 injections 12 hours apart), with a total dose of 500 µg. Twenty-three embryos collections were conducted after two superovulation protocols, the first with 8 injections 12 hours apart and decreasing doses (n = 11, group 1) and the second 3 injections at constant doses 24 hours of interval (n = 12, group 2). The average number of lutea corpora obtained is 9.1 per cow for group 1 and 10.4 in group 2. A total number of 140 embryos were collected with an average of 6.08 per cow. Among the embryos collected, 46 and 56 per cent were transferable respectively in group 1 and 2. A little quality gain was recorded in group 2 with an average transferable embryo of 3.8 against 2.2 for group1. In our study we observed the same efficacy for both treatments with a little quality improvement (almost one transferable embryo) when reducing the number of injections at the time of superovulation treatment, the same gain minimal in elite cows can have a significant economic impact.

Keywords: cattle, embryo production, pituitary extracts, superovulation.

Introduction

Improving fertility is a major concern and a priority for the cattle breeder aiming at optimizing the production potential of his animals. Much effort has been focused on genetic improvement and the selection of more productive animals (Schutz and Pajor, 2001). This benefit brought by the selection had a negative impact by decreasing fertility. Barbat *et al.* (2005) report that in Prim 'Holstein, 30 to 50% of the drop in success rate at first insemination is due to the genetic improvement built around the increase in milk production. In Algeria the success rate at first insemination varies from 50% (Ghozlane *et al.*, 2006) to 18.16% (Ghozlane *et al.*, 2010).

Embryo transfer represents a very good alternative to take advantage of both ascendants genetic potential. The selection occurring at father side as well as at mother side, the use of local breed rustic and disease-resistant recipients and the birth of calves with high genetic potential adapted to the country conditions will result in rapid multiplication of cattle with interesting production potential.

Acquired knowledge in the field of ovarian physiology and follicular growth mechanisms alongside with sonography development (Grizelj *et al.*, 2013), have helped develop the hormonal treatments in order to limit the waste of follicles by atresia. It is to know that 99% of follicular reserve is lost half way, even before maturation (Drion

et al., 1996; Hanzen *et al.*, 2000; Monniaux *et al.*, 1983) which does not help multiply the genetic potential of elite females.

In Algeria few works have been carried out in the field of bovine embryos production in cattle. An average of 4 embryos per cow has been recorded in 2003 in a trial at station (Adel, 2003). In autochthonous bovine populations the first results have been achieved in 2004 with an average of 5 embryos per cow of which 2.33 were transferable (Ferrouk *et al.*, 2008).

However, the large variability of embryos production results is a limiting factor for several reasons. Indeed a cow subjected to a treatment may give from 0 to 50 embryos. Nibart (1991) reports that 10 to 20% of the cows do not give any response to superovulation treatment and 24% of collections provide no viable embryo (Mapletoft *et al.*, 2013). This variability is due to intrinsic factors related to the animal (genetic, pregnancy rate...) and extrinsic ones related to the environment like alimentation (Butler, 2000). Among these factors we find the method of superovulation treatment (Kanitz *et al.*, 2002). The multiplicity of injections during a superovulation treatment using FSH (8 injections 12 hours apart) could produce a stressful effect, harmful to the embryos development (Rutledge, 2001). This stress could be decreased by reducing the number of injections. Some studies have investigated the effect of superovulation hormones injections frequency on embryo collection results. Tribulo *et al.* (2012) have performed only a single injection of diluted FSH with hyaluronic acid, but no significant difference was observed compared to the classic protocol with 8 injections.

The aim of the study was to compare cows' response to two superovulation protocols. The first, is the classic one, consists of 8 injections 12 hours apart, the second consists of three injections 24 hours apart using the same solution at constant and higher doses than the classic protocol.

Materials and methods

Animals

The research has been carried out between October 2010 and November 2011 on a dairy farm in the region of Chiffa, 40 km southern Algiers, the capital of Algeria. It is situated in the plain of Mitidja beneath latitude of 36.46°N and a longitude of 2.81°E. The farm owns 120 dairy cows, 30

calves and ten bulls intended for fattening. Ten multiparous cows divided into two groups were included in the study, they are 4 to 6 years old and they have a BCS (Body Condition Score) of 3.5 to 4 estimated through external appearance criteria according to Schröder and Staufienbiel assessment table (Schröder and Staufienbiel, 2006). They show a regular cycle by visual observation of three successive estrus cycles, and do not present any gynecologic pathology at clinical examination. All animals are kept under the same breeding conditions and receive the same alimentation composed mainly of oat hay and concentrate throughout the year. This ration is supplemented by a supply of green fodder with hybrid sorghum during the summer (from June to August and sometimes until September), and forage clover between February and April.

Media and drugs

A commercial solution of pituitary extracts (Stimufol®) was used for superovulation treatment. A phosphate buffer-based collection medium (Euroflush IMV technologies, l'aigle France) and a holding medium (IMV technologies, l'aigle France) consisting of PBS solution supplemented with 4 g/l of BSA (Bovine Serum Albumin), antibiotics and antifungals were used for embryo collections.

Collection equipment

Collection equipment consists of two-ways catheter (Woerlein CH 18) provided with a metal mandrel. For embryos detection, identification and packaging we used an inverted microscope at the lowest magnification, round Petri dishes of 60 mm of Ø (squared) and 35 mm of Ø, and the 0.25 ml straws for embryos packaging. Sanitary jackets have served to protect the collection catheter.

Superovulation treatments and embryos collection

The superovulation treatments were performed over the whole year. A total dose of 500 µg of Stimufol® was injected following a specific protocol. An injection of 5 mg of etiproston (Prostavet, VIRBAC) was performed on the 3rd day of treatment. Inseminations were performed at 48 and 60 hours after prostaglandin injection. Protocols in both groups are implemented between the 9th and the 11th day of the estrous cycle as follows:

Group 1: the cows receive classic superovulation treatment with 8 injections of a 500 µg

pFSH solution diluted into 10 ml of solvent up to 2 injections per day 12 hours apart at decreasing doses (100µg/100µg, 75µg/75µg, 50µg/50µg, 25µg/25µg).

Group 2: the cows receive a superovulation treatment consisting of 3 injections of 500µg pFSH solution diluted into 9 ml of solvent to 24 hours apart and at constant doses of 166.6 µg.

Collections were performed on the 7th day after artificial insemination by cervical route. Each cow was collected at least twice during the study period. The embryos were classified according to the model proposed by Eldsen *et al.* (1978), and then packaged in transparent straws. We considered the embryos of 1st, 2nd and 3rd classes as suitable, the degenerated ones were counted within the whole number of embryos and the unfertilized were put apart.

Statistical analysis

The data were processed thanks to system 7.0 software. The comparison of the ovarian response results and the embryos number and quality between the two protocols (3 vs. 8 injections) was performed with Kruskal-wallis test. Differences were considered significant for $p < 0.05$.

Results

During the 12 months of the study, 23 collections were made in total. The Tab. 1 shows the overall result of the embryos collections in terms of the superovulation protocol.

The ovarian response was low. This is due to the existence of several collections with a number of corpora lutea counts of less than 10 per cow in both groups.

The overall number of embryos in both groups is 140 (62 and 78 respectively for group 1 and 2) and the overall average is 6.08 per cow with a slight advantage for group 2 (5.6 ± 1.2 and 6.5 ± 1.5 respectively for group 1 and 2). Despite the small average of embryos per cow in both groups, the proportions of suitable embryos remain correct particularly in group 2 with 56.41% against 45.16% for group 1.

Even if the difference was not statistically significant, the embryos quality was slightly better in group 2 than group 1 (Tab. 2). In fact, 71 transferable embryos were collected, with 46 versus 25 and averages of 3.83 ± 0.99 versus 2.27 ± 0.94 for group 2 and 1 respectively. About the embryos of class 1, 25 versus 12 and averages

Table 1: Overall result of embryos collections in both groups.

Parameters	groups	
	group1	group 2
Collections	11	12
CL ($\bar{x} \pm \text{SEM}$)	9.09 ± 2	10.41 ± 1.82
Embryos ($\bar{x} \pm \text{SEM}$)	5.63 ± 1.22	6.5 ± 1.49
Transferable ($\bar{x} \pm \text{SEM}$)	2.27 ± 0.94	3.83 ± 0.99
Transferable/collected (%)	45.16	56.41

Collected embryos: Class 1, 2, 3 and 4 (degenerated); SEM: Standard Error of the Mean

Table 2: Comparison of the quality of the embryos of the two superovulation protocols

Parameters			Group 1	Group 2
Fertilized ($\bar{x} \pm \text{SEM}$)	Transferable	Cl 1 et 2	2±0.9	2.83±0.8
		Cl 3	0.27±0.12	1±0.32
	Non-transferable	Cl 4 and degenerated	1.81±0.68	2.16±0.6
unfertilized ($\bar{x} \pm \text{SEM}$)			1.54±0.6	0.83±0.37

SEM: Standard Error of the Mean

of 2.33 ± 0.77 versus 1.09 ± 0.71 were recorded for group 2 and 1 respectively. Regarding the non-transferable embryos (degenerated and non-fertilized), no difference has been observed between group 1 and 2 with averages of 3.36 ± 0.98 and 3 ± 0.75 respectively.

The design of the conventional superovulation protocol imposes the multiplicity of injections, because of the very short half-life of the pituitary extracts used (Demoustier *et al.*, 1988). Thus twice-daily injections for four days (eight injections in total) are necessary, which is very stressful for animals.

We performed 23 collections in two groups of donors to see whether a possible advantage can be brought by reducing the number of injections during the superovulation treatment. In spite of prior gynecologic examination and cyclicity follow-up, the lack of livestock conditions control (especially alimentation) may have affected ovarian reactions and collection results in both groups. In fact the action of alimentation on the ovarian activity and the fertility has been well demonstrated (Butler, 2000).

In the present study, the ovarian response was low with an overall corpora lutea average of about 9.8 (9.09 and 10.4 for group 1 and 2 respectively) (Garcia Guerra *et al.*, 2012; Bo *et al.*, 1991). This could be explained by a poor cattle management, in particular, an energy excess in alimentation. Indeed, a rich intake of fast fermentable food can induce a sub-acute rumen acidosis which is harmful to reproduction (Barnouin and Chacornac, 1992). Furthermore, a fertility decrease of 53% can be caused by a concentrate daily intake between 400 and 500 gr (Poncet, 2002). An increase in the follicular cysts frequency has also been reported by Paragon (1991). This condition has been noticed in our herd and has led on several occasions to postpone superovulation treatments.

In our work the comparison of the ovarian reaction as well as the recoveries results between the two protocols (8 vs. 3 injections) showed no significant difference. The same finding was reported by Purwantara *et al.* (1994), Donaldson (1990) as for him has obtained the same mean number of transferable embryos with 5.5 and 5.3 respectively for the protocols with 8 and 3 injections. Other authors have investigated the effect of reducing the number of injections by comparing the results of multiple intramuscular

injections protocol to the one with a single subcutaneous injection (Misra *et al.*, 1992; Hockley *et al.*, 1992; Bo *et al.*, 1994; Kelly *et al.*, 1997). These studies show the same efficiency for both protocols. In our work, we observed a slight gain in collected embryos quality in favor of group 2 (the protocol with 3 injections). This could be explained by the reduction of the stress effect imposed by superovulation treatment by reducing the number of injections.

Indeed stress deleterious action on fertility has been reported by several authors (Putney *et al.*, 1988; Nibart, 1991; Dobson *et al.*, 2001) which is most often materialized either by delayed development by the embryos degeneration. According to Nibart (1991), the occurrence of any stress before or during a superovulation treatment leads to an increase in the ACTH secretion and prevents the appearance of the LH peak. Thus it promotes the formation of cysts and poor quality ova.

Conclusions

A superovulation protocol based on 3 injections 24 hours apart of pituitary extracts (Stimufol®) could replace the conventional protocol (8 injections 12 hours apart) in cows. This 3 injections protocol has the advantage to reduce the constraints on the animal and on the operator.

A slight improvement of embryos quality has been recorded in favor of the 3 injections protocol. An optimization of the experimentation conditions (increasing the livestock size and the number of embryos collections, a better control of the breeding conditions) could highlight this qualitative improvement.

These findings may have a direct impact on the cost of embryos production in cows. On one hand the reduction in injections number reduces considerably the cost of veterinarian's displacements during the superovulation. On the other hand the possible improvement of the embryos quality (following the reduction of the stress imposed by injections multiplicity) increases donors yield.

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