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STUDYING THREE THERAPEUTIC METHODS FOR REPEAT BREEDER DAIRY COWS IN ZANJAN PROVINCE DURING SUMMER SEASON

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ABSTRACT

One of the problems in the dairy industry is the reduced fertility of cows in summer due to the premature death of the fetus. This study was conducted in the summer of 2014 at two dairy farms of Zanjan province. The insemination of all cows in the herd was administered based on observed estrus and a routine program of morning and afternoon. 200 RB cows were randomly divided into four groups of 50 animals in this study:

In the first group a dose of PGF2α was administered along with insemination, simultaneously. In the second group a dose of GnRH was injected along with insemination, simultaneously and it was repeated on days 5 and 11 after insemination. In third group Metricim ointment was injected into the cows' uterus a day after insemination. In the fourth group: no drug was injected (control group). The animals which did not show estrus 40 to 50 days after insemination, their pregnancy was diagnosed by rectal examination. The results showed that the fertility rate in the first, second, third and fourth groups are 18%, 24%, 44%, 20%, respectively. The difference between the first group compared with the second and fourth groups is not significant. Also, it is not significant between two and fourth group, but it is significant between the third group compared with the first, second, and fourth groups.

Therefore, it can be concluded that due to thermal stress, the administration of PGF2α or GnRH simultaneously with AI can't improve fertility in anestrous cows.

Keywords: Repeat Breeder, Diary cow, Artificial insemination

INTRODUCTION

In repeat breeding problem the cows show signs of estrus and insemination, but return to repeat breeding again. If the process repeats more than three times the cattle are called an estrous cow (Youngquist and Threlfall, 2006). Causes include: 1) Uterine adhesion and infections, especially endometriosis, 2) Using infertile bulls: artificial insemination must be used in this case, 3) Inappropriate time of AI, which must comply with the AM and PM rules, 4) Feeding: lack of minerals and energy and protein deficiency, 5) Oviduct blocking: using the PSP test to solve the problem, 6) Anatomic deficiency should be considered in heifers, 7) Anovulation or delayed ovulation, 8) Luteal deficiency

Ovulatory disorders: in cows the ovulating is unusual. Seven hours after static estrus, LH surge is occurred, ovulation occurs after 28 hours of LH surge, i.e. 12 hours after the end of estrus the ovulation occurs. Sometimes the egg is not released, and/or has a delayed release or oocyte becomes aging and unable to grow normally. Ovulation disorders are due to the following reasons: endocrine deficiency or imbalance, lack of the production of a hormone receptor in target tissues, and mechanical factors (Arthur *et al.*, 1996).

Delayed ovulation: It may occur about 15% of cases. This is one of the reasons of the Repeat Breeder syndrome. All estrous cows should be examined 24 hours later in order to graph follicle intact. This is due to the lack of LH and Gonadotrophin that causes delayed the egg release. For treatment: GnRH is injected 3 to 6 hours before insemination that causes the rapid rise in FSH and LH and reach their peak point within 30 to 60 minutes, then return to their before injection amounts within 4 hours. Alternatively, the AI is conducted two times; once during estrus and another one is administrated after 24 hours (Youngquist and Threlfall, 2006).

Anovulation: It is clinically similar to the delayed ovulation, i.e. the cattle become estrous and show signs of estrus, but the ovulation doesn't occur despite AI. The similar condition occurs in the first month after calving that is cured spontaneously. In this case, a follicle grows up to ovulation, but the ovulation does not occur and the follicle wall becomes luteinized. These kinds of follicles usually dwindle spontaneously. In this case, probably a

normal ovulation occurs following the next estrus. The follicle that has no ovulation may be mistaken with vacuolar corpus luteum (CL). These follicles have the same amounts of non-cystic CL progesterone. Luteinized follicle remains 17-18 days before dwindling. In this case, the ovary is rounded and smoother rather than a thick and irregular shape.

Treatment is conducted to ensure the future of ovulation is done in the next intercourse; so, HCG or GnRH is injected 3 to 6 hours before insemination or at the time of insemination (Arthur *et al.*, 1996).

Luteal deficiency: The corpus luteum of pregnancy does not suffice to provide progesterone. Progesterone is the main hormone of pregnancy, and luteum corpus provides it by the day 150 of pregnancy. Therefore, if the corpus luteum does not provide enough progesterone it is more likely to luteal failure. Luteal deficiency is one of the causes of the Repeat Breeder syndrome. One dose of GnRH is injected 11 to 13 days after insemination to prevent such a situation because it is a luteotrophic. Another way is to use the CIDR after 5 days of insemination into the vagina for 2 weeks.

In this interval, the pregnancy and binding embryo to endometritis has not been detected. In cows, some materials such as BTP and zygotene are secreted on days of 14 to 16 of embryonic life, which prevents abortion. Also, nutrition has a positive effect on the quality and diameter of the produced follicles. So, the corpus luteum will have a large diameter and an increased progesterone production by a good nutrition (Arthur *et al.*, 1996). With the adjustments of environmental temperature and focusing on the developmental biology of embryonic, destructive effects of high environmental temperature on fertility can be reduced (De Rensis and Scaramuzzi, 2003). In the three recent studies, it has shown that administration of prostaglandins along with insemination in the summer will cause an increased fertility by 15.2%.

In a study by Lopez et al. On the effect of prostaglandins during the summer it showed that prostaglandins increase the rate of ovulation by 4.2 times and the rate of double- ovulation by 2.6 times and a significant increase in pregnancy rates (Lopez-Gatius *et al.*, 2004).

MATERIALS AND METHODS

In this study, 200 RB cows were randomly divided into four groups of 50 animals: In the first group a dose of PGF2α was administered along with insemination, simultaneously. In the second group a dose of GnRH was injected along with insemination, simultaneously and it was repeated on days 5 and 11 after insemination. In third group Metricim ointment was injected into the cows' uterus

a day after insemination. In the fourth group: no drug was injected (control group). The animals which did not show estrus 40 to 50 days after insemination, their pregnancy was diagnosed by rectal examination.

Gonadorelin: 10 ml vial of Gonadorelin with the commercial name of vetarolin from the Aboureihan pharmaceutical company was used.

Cloprostenol: 10 ml vial of Cloprostenol from Aboureihan pharmaceutical company was used, which is in the synthetic PGF2a form and has all its effects.

Metricim: Each syringe contains 500 mg Cephapirin (Benzathine).

RESULTS AND DISCUSSION

The animals which did not show estrus 40 to 50 days after insemination, their pregnancy was diagnosed by rectal examination. Finally, the following results were obtained:

Table1: Pregnant cows, nonpregnant cows diagnosed, and fertility rate

	Pregnant cows	Non-pregnant diagnosed	Fertility rate (%)
Group1	**41**	**9**	**18**
Group2	**38**	**12**	**24**
Group3	**28**	**22**	**44**
Group4	**40**	**10**	**20**

The results were statistically analyzed (based on chi-square test) and it was found that: The difference between the fertility rates of the first and second group was not significant. The difference between the fertility rate of the first and fourth groups was not significant. The difference between the fertility rate of the second and fourth groups was not significant. The difference between the fertility rates of the third group compared with first, second, and fourth groups, was significant.

Therefore, it can be concluded that due to thermal stress, the administration of GnRH or PGF2α simultaneously with AI cannot improve fertility in anestrous cows. Furthermore, the most common cause of infertility in dairy cattle is the acute and chronic endometritis which is cured using intrauterine antibiotics.

Insemination pregnancy rate in dairy cows is reduced in hot season compared with the cold season by 20-30 % (Joyce *et al.*, 2001). Several factors are involved in this case, the most important of which is the effect of the increased temperature and humidity that cause the reduction of steroid production capacity in single and granulosa layers, and finally result in decreased concentration of blood estradiol as well as estrous.

Most studies on the importance and role of prostaglandins in ovulation process have been conducted in the laboratory and fewer studies have been done on the farm.

Yet, using the intravenous cloprostenol simultaneously with insemination, the

pregnancy rate has been improved by 15.2%. Also, Yuniz and Lopez in 2004 also used coincided PGF2α and insemination in the summer and could improve ovulation rate by 4.2 times (Lopez-Gatius *et al.*, 2004).

But in cows without thermal stress and high reproductive performance that result was not obtained. On the other hand, although some percentage of infertility is attributed to the ovulatory problem, PGF2α and GnRH, which was expected to improve ovulation and pregnancies in the animals with a low reproductive rate, had no favorable result that inconsistent with the results of the study conducted by Lopez et al.

This study has been conducted to reveal the clinical role of prostaglandin in the increased probability of ovulation in the animal husbandry industry. The results obtained by the study didn't show any meaningful treatment effect of PGF2α compared with the control group.

In summary, several factors were changed in the summer, which led to the decline of fertility that the follicular growth and ovulation has a special place in case. The use of hormones such as Gonadorelin and PGF2α was suggested in more recent studies in order to remove follicular development and ovulation disorders

On the other hand, it has been claimed that one of the ways to cope with heat stress is the administration of progesterone after insemination. The administration of GnRH or HCG on days 5 and 11 after insemination was conducted to induce the formation of secondary corpus luteum, which occurred with the ovulation or luteinization of the grown follicles to produce more progesterone in order to improved pregnancy rates.

It was found in this study that the administration of GnRH on day 5 to day 11, causing the formation of more corpus luteum, more progesterone production and higher pregnancy rate compared with its administration on day 11. In general, it seems that the most important reasons for the RB problem on farms with a regular estrus detection, an experienced artificial inseminator, and the good quality sperms are subclinical infections (subclinical endometritis) because delayed ovulation and lack of ovulation form a very small percentage of RB causes that need to prescribe medicines as GnRH, HCG and PGF2α that cause ovulation at the time of insemination.

On the other hand, the heat stress is the most important causes of RB by effecting on follicle growth and the production of the follicles with a low fertility power. Heat stress, at least in Iran, doesn't reach to the extents that make a serious problem with ovulation.

Low quality of oocytes in heat stress and the conditions of uterus environment for the sperm and the zygote, are some factors, which cause the reduced pregnancy in summer and increased rate of RB, so in situations like this case other ways of coping to heat such as cooling, ventilation, use of mist are and instead recommended instead of the use of hormones. Approximately 25% of bovine embryos die in the first three weeks of their life (Peters, 1996). Continuous production of progesterone by an active and healthy corpus luteum is vital at the beginning of pregnancy, and the loss of the corpus luteum is the leading cause of premature abortion.

The use of GnRH is as a support to pregnancy on insemination day to improve the performance of RB cattle, which has been one of the main reasons for the use of this hormone during the past 25 years. Science reasons behind the use of GnRH in ovulation induction at the right time to time as well as luteinization induction that is expected to obtain a successful improvement in fertilization and embryo surviving. In a review of the scientific literature published on the efficiency of GnRH used during estrus (Stevenson *et al.*, 1990) it has reported that, in the 14,000 tested cattle, 6 to 7% improvement upon the first insemination was occurred in RB cattle. The reports on these kinds of improvements with the same number of cattle in other trials show that why the hormone has not similar results in low numbers of cattle.

Lean et al., are the main exponents of studying techniques usage about the cow reproductive (Beckett and Lean, 1997- Burton and Lean, 1995- Morgan and Lean, 1993).

The Meta analysis provides the obtained data for the studies on the cattle reproduction, since the published articles results are usually extremely inconsistent and the guidelines presented in these papers by various research groups are hardly comparable to each other. Therefore, these methods (e.g., Meta analysis) allow us to gather information about various studies, to separate the inconsistent and to merge others in order to increase the precision and power of the work and finally it allows us to examine the obtained results (Morgan and Lean, 1993). The mentioned authors conducted a Meta analysis about 40 different experiments which was conducted on 19019 cattle in 27 published papers.

In this experiment, GnRH or its analog was administered at the time of insemination in cattle. Mantel Haenszel analysis showed that the use of GnRH and its analogues (Buserelin) increases pregnancy rates in all groups by 12.5%. Nevertheless, the effects of the drug were higher in RB cattle by 22.5%.

Relative probability of reproductivity (RR), i.e. the risk of pregnancy in cows treated with respect to the likelihood of cattle controls (RR for cattle control equals 1) in one of the tests where we injected GnRH on day of insemination in dairy cattle (Drew and Peters, 1994) was equivalent to an increase of 6.1% in the total fertility rate which was not significant.

These results are very close to the Mee et al. (1990) study. Also, there was a significant negative correlation between the relative improvements in pregnancy rates compared with cows in the same herd in the control group. In other words, the worse herd background, the more improvement in reproductivity using GnRH.

In another method first has been used by McMillan et al (1986) in New Zealand, GnRH or Buserelin was injected into cattle within first, third, fourth, Sixth, seventh and / or tenth, eleventh and thirteenth days of insemination. In these experiments, only the injections between the eleventh and thirteenth days cause a significant improvement in pregnancy rates. This type of treatment method was conducted in many countries. In England, the results from two experiments showed an improvement of 9.4% and 12% (Drew and Peters, 1994-Kaim *et al.*, 2003)., whereas there was no difference in larger trials conducted in Australia and Ireland (Jubb *et al.*, 1990). Look at the selected published data on the effects of GnRH analogues on pregnancy rates in cows that were injected on days 11 to 13 after insemination. Observe the percentage difference among the treated cows in the last column. These conflicting data prompted us to conduct a Meta analysis about the published articles on the use of GnRH analogues on days 11 to 14 after the first insemination. We analyzed the results of 19 separate studies in the paper. The Odds ratio (relative probability of pregnancy between the treated group and the control group) in any experiment was compared and significant differences between different experiments were appeared that its range was ariable from zero to 22 percent.

Logistic regression showed that the response to GnRH treatment depends on factors such as the cow type (dairy or beef), calving number, using a method for synchronization (scheduled or natural), the method for pregnancy diagnosis, as well as the effects of each individual study. All the variables were included in the analysis, but only 6 of the 14 articles had the capability to analyze in which there were 2541 cattle of total 10,845. In this statistical group a significant increase in productivity rates was observed in treating cows (the probability rate was 1.33). Although such an analysis of obtained data in cattle farms provides a

support for practical and routine use of GnRH for, a kind of pharmacological-physiological explanation about the mechanism of function is necessary to present a perfect evaluation of its usage. In these studies, a stage in which GnRH was administered (days 11 to 14 after insemination) is important because this is when the maternal recognition of pregnancy occurs. At this time interferon tau factor which is an Anti-luteolytic is secreted by the embryo (Arthur *et al.*, 1996).

It has been shown that GnRH injection in non-pregnant cows in the mid and late life of CL stimulates the CL to secrete progesterone (Arthur *et al.*, 1996). Nowadays, it has been clear that cows have 2 or 3 follicular wave in each estrous cycle (Webb *et al.*, 1991).

Depending on the state of follicles at any time, treatment with GnRH during the luteal phase of causes decomposition, luteinization, or ovulation and the luteinization. These changes, apart from stimulating the secretion of progesterone, also reduce the production estradiol- 17–Beta. This reduction causes inhibition of oxytocin receptors production, and as a result, the production and secretion of PGF2α are avoided (Mann and Picton, 1995). Therefore, it is expected that the reduction in estradiol secretion causes the reduction of the destructive mechanisms of CL and abortion doesn't occur. It was concluded that GnRH causes delay or attenuation of destructive messages of CL, so that the fetus has more time to increase its activities against the destruction of the CL. Cows with three follicular waves, GnRH treatment on days 11 and 14 coincides with the second follicular wave increase (Mann and Picton, 1995). Therefore, at this time interval the estradiol rate is high in blood. Estradiol concentration in cattle with two follicular waves is low at this time. So, it is interesting to determine the difference between two-wave and three-wave cows' response to GnRH. This argument can be one of the reasons of inconsistent results, although this is a hypothesis yet. Using GnRH following insemination, especially in cows suffered from heat stress and body weakness has been reported (Kaim *et al.*, 2003).

In conclusion, the data show that treatment with GnRH is beneficial following the insemination, but the environmental and physiological variables which have an effective role in response to the treatments have not been evaluated yet.

REFERENCES

[1] Youngquist RS and Threlfall WR, Current Therapy in Large Animal Theriogenology, Elsevier Health Sciences, 2006.

[2] Arthur GH, Noakes DE, Pearson H and Parkinson TJ, Veterinary Reproduction and Obstetrics, WB Saunders, 1996.

[3] De Rensis F and Scaramuzzi RJ, Heat Stress and Seasonal Effects on Reproduction in the Dairy Cow—a Review, Theriogenology, 60(6), 2003, 1139-1151.

[4] Lopez-Gatius F, Yaniz J, Santolaria P, Murugavel K, Guijarro R, Calvo E and Lopez-Bejar M, Reproductive Performance of Lactating Dairy Cows Treated with Cloprostenol at the Time of Insemination, Theriogenology, 62(3), 2004, 677-689.

[5] Joyce IM, Pendola FL, O'Brien M and Eppig JJ, Regulation of Prostaglandin-Endoperoxide Synthase 2 Messenger Ribonucleic Acid Expression in Mouse Granulosa Cells During Ovulation 1, Endocrinology, 142(7), 2001, 3187-3197.

[6] Peters A, Embryo Mortality in the Cow, Animal Breeding Abstracts (United Kingdom), 1996.

[7] Stevenson JS, Call EP, Scoby RK and Phatak AP, Double Insemination and Gonadotropin-Releasing Hormone Treatment of Repeat-Breeding Dairy Cattle, Journal of Dairy Science, 73(7), 1990, 1766-1772.

[8] Beckett S and Lean I, Gonadotrophin-Releasing Hormone in Postpartum Dairy Cattle: A Meta-Analysis of Effects on Reproductive Efficiency, Animal reproduction science, 48(2), 1997, 93-112.

[9] Burton N and Lean I, Investigation by Meta-Analysis of the Effect of Prostaglandin F2 Alpha Administered Post Partum on the Reproductive Performance of Dairy Cattle, Vet Rec, 136(4), 1995, 90-94.

[10] Morgan W and Lean I, Gonadotrophin-Releasing Hormone Treatment in Cattle: A Meta-Analysis of the Effects on Conception at the Time of Insemination, Australian veterinary journal, 70(6), 1993, 205-209.

[11] Drew S and Peters A, Effect of Buserelin on Pregnancy Rates in Dairy Cows, Vet Rec, 134(11), 1994, 267-269.

[12] Kaim M, Bloch A, Wolfenson D, Braw-Tal R, Rosenberg M, Voet H and Folman Y, Effects of Gnrh Administered to Cows at the Onset of Estrus on Timing of Ovulation, Endocrine Responses, and Conception, Journal of Dairy Science, 86(6), 2003, 2012-2021.

[13] Jubb T, Abhayaratne D, MALMOS J and Anderson G, Failure of an Intramuscular Injection of an Analogue of Gonadotrophin-Releasing Hormone 11 to 13 Days after Insemination to Increase Pregnancy Rates in Dairy Cattle, Australian veterinary journal, 67(10), 1990, 359-361.

[14] Webb R, Gong J, Law A and Rusbridge S, Control of Ovarian Function in Cattle, Journal of reproduction and fertility. Supplement, 45(1991, 141-156.

[15] Mann G and Picton H, Ovarian and Uterine Effects of a Single Buserelin Injection on Day 12 of the Oestrous Cycle in the Cow, J. Reprod. Fertil. Abstr. Ser, 1995, 61.