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Effect of Temperature on Embryos Obtained During Embryo Transfer Process

[1]E. Kabourkova, [1]P. Slama and [2]H. Corten
[1]Department of Animal Morphology, Physiology and Genetics Mendel University in Brno, Brno, Czech Republic
[2]CAH Dronten University of Applied Science, Dronten, Netherlands

Abstract: The objective of this manuscript was to evaluate the effect of ambient temperature on the uterus flushing out during the embryo transfer process. The study was carried out on dairy farms and was performed from January 7 to November 19, 2010. The milking cow donors flushed out during hot weather were compared with donors that were flushed out during cold weather. It focused on the number of embryos obtained and the ambient temperature. The number of embryos obtained was recorded during the embryo transfer process. The three groups of milking cows were measured during high ambient temperature, medium ambient temperature and low ambient temperature while they were in heat. The experiment was performed on the Holstein breed of dairy cow. The experiment focused on the viable embryos present in the flushed liquid. The number of embryos obtained while the cows were in heat fluctuated with the difference in ambient temperatures, being the lowest during high ambient temperature. The number of embryos was high during medium ambient temperature and during low ambient temperature. Low ambient temperature had no significant effect on the number of embryos. Because high ambient temperature has a significant negative effect on the number of embryos obtained, it seems necessary to protect milking cows that are in heat against high ambient temperatures.

Key words: Milking cow, flushing out, heat, superovulation, ambient

INTRODUCTION

Embryo transfer is a process that presents a tool for genetic improvement. Due to the embryo transfer technique there is potential to create genetic improvement twice as fast as by using artificial insemination alone. The embryo transfer process for cattle has been used for several years as a method of multiplying imported exotic breeds, since the early 1970‘s. The cows considered for the embryo transfer process are first evaluated and then only high value cows are selected. The donor cows of a particularly fine pedigree are treated with hormones to increase the number of eggs released during ovulation. The goal is to get several mature eggs rather than a single egg,which would normally develop each month. The process is known as superovulation.

The selected donor cows are treated with a gonadotropin called the Follicle Stimulating Hormone (FSH). This hormone is administered following estrus while a functional Corpus Luteum (CL) is in the ovary. As a result of the treatment, multiple follicles should be developed in the ovaries of the donors. Then multiple eggs are released during estrus. In order to bring cows in estrus, a prostaglandin such as Lutalyse is injected on the fourth day of the FSH treatment schedule. The prostaglandin causes CL regression and estrus occurs approximately 48 h later. The following superovulatory treatment requires donor cow observation for signs of estrus. Cows that were superovulated do not display estrous behavior as clearly as untreated cows. The donor cows are inseminated by semen from any bull 12 h after the onset of estrus and again twelve hours later. The embryos are flushed out from the donor ’s uterus seven days after the insemination (Linares, 1981).

The embryos obtained may then be implanted into the recipient cows whose estrous cycle is at the correct receptive stage-usually as a result of hormonal manipulation. The excess embryos obtained can be stored using cryopreservation. Usually, the extra embryos obtained are frozen. Therefore, a donor cow does not have to go through the ovarian stimulation and the egg collection each single cycle. Frozen embryos have become

Corresponding Author: E. Kabourkova, Department of Animal Morphology, Physiology and Genetics Mendel University in Brno, Brno, Czech Republic

easily transferable and it is possible to sell them. Anyone may purchase the embryos obtained during the embryo transfer process from anywhere in the world and transfer them into their own recipient cows.

The number of embryos obtained during the embryo transfer process has a huge influence on the farm economy. The variability in the superovulatory response continues to be one of the most frustrating problems with embryo transfer in cattle. The cost of the embryo transfer process is around €700 (according to Co. ETS. Holland, 2011). The average of the embryos obtained per a cow is 5.26 (Lazarevic and Miscevic, 2001). When a farm calculates the expenditure for an embryo, the cost is approximately €133 per embryo. However, the price of each embryo depends on the value of the donor breed and not on the cost of the embryo transfer process and the number of embryos obtained.The value of an embryo is from €50-2.000. Nevertheless, the number of embryos obtained by flushing out is different every single time. There has been uncertainty about the effect of ambient temperature on the number of embryos flushed out from a donor. The low number of embryos obtained has an adverse effect on farm economy. It also affects the efficiency of veterinarians and other specialists who are involved in embryo transfer. Farmers try to improve the conditions of the flushing out process in order to improve their productivity and profitability.

The production of embryos by superovulation is often reduced in periods of heat stress. The associated reduction in the number of transferable embryos is due to the reduced superovulatory response, lower fertilization rate and reduced embryo quality. Heat stress can compromise the reproductive events required for the embryo production by decreasing expression of estrus behavior, altering follicular development, compromising oocyte competence and inhibiting embryonic development (Hansen *et al*., 2001). Reproduction suffers as a result of heat stress effect on folliculogenesis and oocyte maturation as well as embryonic development and survival. Under the influence of heat stress the duration and the intensity of estrus are reduced. There is a clear decrease in mobility and other manifestations of estrus (Kanitz *et al*., 2002). Luteinizing hormone secretion from the pituitary gland does not appear to be impaired in animals exposed to high ambient temperatures (Gwazdauskas *et al*., 1981). In contrast, a clear reduction in the pulse and amplitude of luteinizing hormone release was observed in cows exposed to heat stress (Kanitz *et al*., 2002). The decrease in productivity and negative reproductive effects occurring during periods of heat stress and those periods following reduces the profitability of dairy operations, making heat stress abatement practices cost effective and advantageous.

Heat stress is a general name for several medical conditions such as heat exhaustion, heat cramps (muscle pain or spasms) and heat stroke caused by being in hot areas (Berman, 2005). Heat stress is commonly assessed by the temperature-humidity index, the sum of the dry and the wet bulb temperatures. There is a very strong relationship between air temperature and heat stress.

Evaporative cooling is the principal mechanism for heat dissipation in cattle at high temperatures. It is influenced by humidity, wind speed and by physiological factors such as respiration rate, density and performance of sweat glands. The failure of homeostasis at high temperatures may lead to reduced productivity (Blackshaw and Blackshaw, 1994). In severe cases cows may die from extreme heat, especially when it is combined with other stresses such as illness or calving (Berman, 2005).

Heat stress in cattle occurs when the heat gain becomes greater than the ability of the animal to lose heat. The total heat load is made up of the body heat from metabolism plus environmental heat. Heat stress causes reduced productivity in dairy cattle herds (Gwatibaya *et al*., 2007). The bovine thermal comfort zone is from-13°C till+20°C (Kadzere *et al*., 2002). According to Dragovich (1980), the lower limit is 4°C and according to Broucek *et al*. (2009), the upper critical air temperature is >24°C. The optimal body temperature of cows is between 38.4 and 39. 1°C. At temperatures above 24°C cows suffer from heat stress: their health status and zootechnical performance are affected (Kadzere *et al*., 2002). During hot weather the reproductive proficiency of lactating dairy cows is greatly diminished. Heat stress can reduce follicle and oocyte maturation (Hansen and Arechiga, 1999). As a result, the number of the embryos obtained could be low.

The increase in body temperature caused by heat stress has a direct adverse effect on cellular function. For instance, elevated temperatures reduce the proportion of embryos that can continue their development. The magnitude and geographical extent of this problem is increasing because improvements in milk yield have made it more difficult for cows to regulate body temperature during warm weather (Hansen, 2007). Lactating dairy cows create a large quantity of metabolic heat and accumulate additional heat from radiant energy. The heat production and accumulation coupled with compromised cooling capability due to environmental conditions causes heat load in cows to increase to the point that body temperature rises,intake declines and ultimately the cow's productivity declines. Body heat production is associated with milk yield and this influence increases as metabolic processes, feed intake and digestive requirements increase with yield (West, 2003). The goal is to obtain as many embryos as possible. Therefore, the goal of this project was to evaluate the effect of ambient temperature on the uterus flushing out during the embryo transfer process.

MATERIALS AND METHODS

The study was carried out on dairy farms. In total, cows were included. The animals were divided into three groups by the level of ambient temperature. These groups were observed from January 7 to November 19, 2010. Two veterinarians flushed out the uteruses of donor cows selected during the embryo transfer process. The experiment focused on the embryos present in the flushed liquid. The flushed liquid was strained through a sterile sifter. After that it was observed under a microscope by trained assistants. Only the viable embryos were chosen and counted. The process of the uterus flushing out was done by two different veterinarians. However, the technical processes were absolutely uniform. Both of them had a similar work flow for the whole process of the uterus flushing out and the same tools were used.

Different ambient temperatures were chosen because the research was focused on ambient temperature's effect on embryos obtained during the embryo transfer process. The main research question was: What is the effect of ambient temperature on the uterus flushing out during the embryo transfer process?

The established hypotheses were: The number of the embryos obtained is not different during the high/medium/low ambient temperature (H_0) and the number of the embryos obtained is different during high/medium/low ambient temperature (H_1). The donor cows for the uterus flushing out process were selected and divided according to the level of ambient temperature into three groups, with eleven members per a group. The ambient temperature was measured while the donor cows were in heat. The donor cows in group A were in heat during high ambient temperature. The donor cows in group B were in heat during medium ambient temperature. The last group, group C, was formed by donor cows that were in heat during low ambient temperature. Group B was used as the control group for groups A and C.

The experimental groups were marked by the letters A, B and C. Group A represented the level of high ambient temperature which was ≥ 24°C (Broucek *et al*., 2009) in this experiment. Group B represented medium ambient temperature level which was between >4 and <24°C. The last group, Group C, represented low ambient temperature which was = 4°C (Dragovich, 1980).

All donor cows from the three groups were inseminated by bull semen 12 h after the onset of estrus and then again twelve hours later. On the 7th day after the insemination the uterus flushing out was conducted. All results of the flushing out were recorded in the MS Excel spreadsheets. Data were collected in MS Excel and analyzed by the SPSS Statistics 17.0.

RESULTS AND DISCUSSION

With regards to the results, the milking cows produced more embryos ($p<0.05$) during low ambient temperature than the cows during high ambient temperature. Furthermore, the cows produced more embryos ($p<0.05$) during medium ambient temperature than the cows during high ambient temperature. High ambient temperature had a significant ($p<0.05$) negative effect on the number of embryos obtained. The trend mentioned above is noticeable in Fig. 1. Figure 1, shows the distribution of the population in every ambient temperature group (high, medium and low). Every single segment illustrates the range of distribution. Furthermore, Fig. 2 shows a graphical representation of the distribution

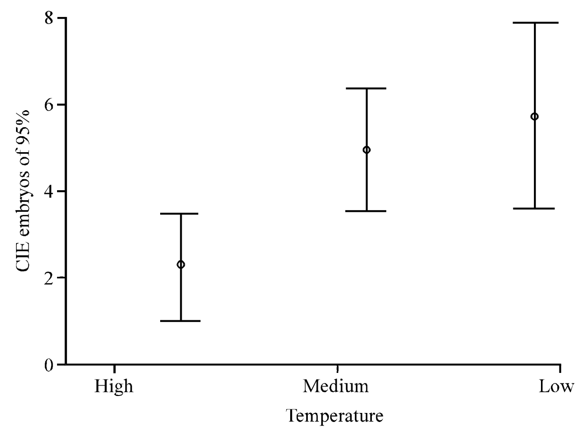

Fig. 1: The distribution of the viable embryos obtained in high, medium and low ambient temperature

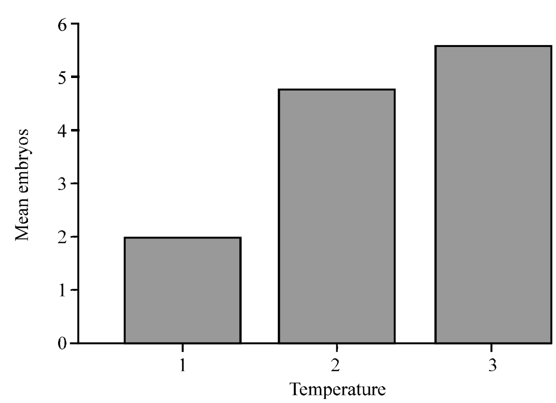

Fig. 2: The distribution of the viable embryos obtained in high (1.00), medium (2.00) and low (3.00) ambient temperature

Table 1: Single numbers from 1-12 and total number of viable embryos obtained during high (1.00), medium (2.00) and low (3.00) ambient temperature

	Embryos obtained										
Temperature	1	2	3	4	5	6	7	8	11	12	Total
1.00	7	2	0	1	0	0	1	0	0	0	11
2.00	0	2	2	1	1	3	0	2	0	0	11
3.00	1	0	2	1	3	1	1	0	1	1	11
Total	8	4	4	3	4	4	2	2	1	1	33

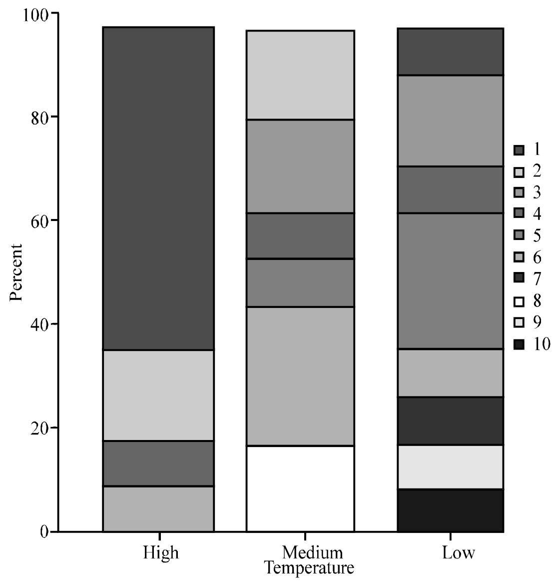

Fig. 3: The frequency of distribution of the viable embryos obtained from 1-12 pieces (%) in high medium and low ambient temperature

of the population in every temperature group. Temperature Group No. 1 means high ambient temperature, Group No. 2 represents medium ambient temperature and Group No. 3 is low ambient temperature. Table 1 represents an overview of the number of embryos obtained during high, medium and low ambient temperatures.

Figure 3 represents, the same frequency of distribution as the previous figure. Each color represents the actual number of embryos obtained. Obviously, during high ambient temperature just one embryo was the most frequent result. On the other hand, during low ambient temperature twelve embryos were obtained during the uterus flushing out process.

Due to the many projects which were made on this topic, the negative influence of high ambient temperature was expected. This result is consistent with the data reported by Hansen *et al.* (2001) who reported that the production of embryos by superovulation is often reduced in periods of heat stress (climatic factors that may influence the degree of heat stress include: temperature, humidity, radiation and wind (Hansen *et al*., 2003) and the associated reduction in the number of transferable embryos is due to the reduced superovulatory response, lower fertilization rate and reduced embryo quality. Furthermore, it is reported that the success of *in vitro* fertilization procedures is reduced during the warm periods of the year and heat stress can compromise the reproductive events required for the embryo production by decreasing expression of estrus behavior, the altering of follicular development, the compromising of oocyte competence and the inhibiting of embryonic development (Hansen *et al*., 2001). High ambient temperature reduces embryo quality. The superovulation result is highly dependent on the kind of gonadotropin used and on the frequency of its application. On the other hand, no significant influence on the embryo yield could be noted, regarding the day of the cycle on which the treatment is started (Benyei *et al*., 2003). Concerning the yield of embryos, the age of the donor also has no significant impact on the results.

The low ambient temperature had no significant ($p>0.05$) effect on the embryos obtained. Based on the outcome, the zero hypothesis (H_0) had to be rejected and at the same time hypothesis number one (H_1) was accepted. The number of embryos obtained is different during high/medium/low ambient temperature (H_1).

CONCLUSION

The number of embryos obtained while the cows were in heat, fluctuated with the level of ambient temperature, being the lowest ($p<0.05$) during high ambient temperature (24°C and higher), high during medium ambient temperature (4.1-23.9°C) and high again during low ambient temperature (4°C and lower). The negative influence of high ambient temperature on the uterus flushing out during the embryo transfer process was expected. As the high ambient temperature had a significant ($p<0.05$) negative effect on the number of embryos obtained, it seems to be necessary to protect milking cows against high ambient temperatures while they are in heat. Low air temperature had no significant effect on the number of embryos obtained. There is no need to protect them during low ambient temperatures. A possible solution for the caretakers of dairy cows could be to select the most suitable ambient temperatures for the time of estrus evocation and the following embryo transfer.

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