Selection of embryo recipients, a real challenge in cows embryo transfer programs

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ABSTRACT

This article comes as a response to a clinical situation that the members of our team found at an embryo transfer program. At the moment of insemination, day 7 after estrus period, a high number of untransferable recipients was found after ovaries ultrasonography. From 54 animals synchronized, just in 5 (9.25 %) cases was performed the embryo insemination. The embryo transfer was performed in cases where a corpus luteum (CL group) higher than 17 mm was found on the ovary, corpus luteum with a cavity of a maximum 10 mm and without a follicle higher than 10 mm on the ovary with the corpus luteum or on the other ovary. The rest of heifers were classified into the next groups: Hollow corpus luteum (HCL Group), 11 cases (20.37%), Lutheal cyst (LC Group), 5 cases (9.25%), No corpus luteum (NCL Group), 4 cases (7.40%), Corpus luteum and follicle (CLF Group), Small corpus luteum (SCL Group). Cystic corpus luteum seems to be a good variant for performing the Embryo Transfer. Even if the level of P4 concentrations in the case of a luteal cyst is highest, the embryo transfer in these cases is with a high level of risk and should be avoided.

Keywords: embryo insemination, corpus luteum, ultrasonography, embryo recipients, cows

INTRODUCTION

Our team members discovered a practical issue at an embryo transfer program and this publication is a response to that situation. After ovaries ultrasonography on the day of insemination, day 7 after estrus period, a high percentage of non transferrable recipients was discovered. The situation encountered by our team at the time of embryos inoculation put the members of the team in the difficulty to perform or not the embryo transfer for a high percent of females. The high price of embryos and the uncertain situation have led them to insert embryos into only a small percentage of recipients and to conduct some research in order to determine which groups of females are amenable to embryo transfer and which are not.

Despite the fact that the regulation of bovine luteal function has been studied for decades, many of the regulatory mechanisms involved remain unknown. We're still a long way from fully comprehending how these intricate processes work together (Kottferová et al., 2014). The corpus luteum (CL) is a temporary reproductive gland that produces progesterone (P), which is

necessary for establishing and maintaining a pregnancy (Schams and Berisha, 2004). The corpus luteum has two physiological morphological types in cattle: compact and with the cavity. A fluid-filled hollow is plainly evident during ultrasound examination (Jaśkowski et al., 2021). Hollow corpora lu-tea, which are not pathological, should not be confused with luteal cysts (Garverick, 1997). Young corpora lutea with an antrum are known as hollow corpora lutea. A luteal cyst and a cystic corpus luteum can be distinguished via an ultrasound scan of the ovaries (Vanholder et. al., 2006, Jankowska et al., 2010).

One of the component procedures in the selection of recipients on the day of embryo transfer is a clinical examination of the ovaries to confirm the existence of the corpus luteum (CL). Several studies have proven that a CL of at least 17 mm in diameter provides a satisfactory conception rate and pregnancy maintenance when the embryo is placed into the ipsilateral horn of the uterus (Yoshida et al., 2016).

Heifers receiving fresh embryos had a lower pregnancy rate if their progesterone (P4) level was less than 1 ng/mL, and previously frozen embryos had a lower pregnancy rate if their P4 concentration was less than 3 ng/mL (Stubbings and Walton, 1986).

The cavity can be seen gradually filling with luteal tissues and eventually disappearing. A cystic CL is defined as a CL with a cavity more than 7 to 10 mm in diameter or a cavity that persists for more than 7 days following AI. Cystic CL has been identified as a possible cause of infertility in cows by several researchers, and many therapies have been offered. Other researchers, on the other hand, believe there is no link between cystic CL and cow infertility (Kito et al., 1986).

The purpose of this study was to present a true circumstance that occurred in an embryo transfer program and to provide a response to similar situations for the embryo transfer teams in cows.

Materials and Methods

An embryo transfer program with frozen embryos imported from Uruguay was launched in September 2021 for 54 Black Angus heifers, aged 17-20 months, weighing between 380-420 kg. The heifers were raised on pastures, in free system, with quality hay and silo during the winter, in Timis county, Romania. On day 1 of the embryo transfer program all heifers were checked by ultrasound and 1 bolus with minerals and vitamins (Obifertile, Obione, France) was administered. The estrus synchronization protocol performed for embryo recipients was by administering i.m. 2 doses of 0.5 µg Cloprostenol (Estrumat, 2 ml i.m, Maravet) at 11-day intervals, days 1 and 11. All heifers were monitored for signs of estrus in the 48-96 h range after the second dose of Cloprostenol, days 13-15. On the 21st day, the 7th day after estrus, embryo transfer was performed. Prior to insemination, a transrectal ultrasound examination was performed to confirm the presence of an active corpus luteum with a diameter greater than 17 mm in the absence of a follicle greater than 10 mm. Embryo insemination was performed in the uterine horn ipsilateral to the corpus luteum. Serum samples were collected to determine progesterone levels on the day of embryo transfer. The transrectal ultrasonography was performed using the portable ultrasonograph Easy Scan , from BCF Technology.

Table 1 Embryotransfer protocol

Day	**1**	**11**	**13-15**	**21**
Maneuvers	TR echography, Estrumate administration	Estrumate administration	Estrus monitorization	TR echography Inseminations of embryos, collection of serum samples

RESULTS AND DISCUSSIONS

An uncommon scenario was discovered during the transrectal ultrasonography check done on day 7 after estrus. The embryo insemination was performed in only 5 (9.25%) of the 54 synchronized animals. The embryo transfer was done in cases where the ovary had a corpus luteum (CL group) bigger than 17 mm, with a cavity smaller than 10 mm, and no follicle measuring more than 10 mm on the ovaries. The remaining heifers were divided into the following groups:

- Hollow corpus luteum (HCL Group), 11 cases (20.37%), Corpus luteum bigger than 17 mm in diameter with a cavity between 10-15 mm, luteal tissue thicker than 3 mm.

- Luteal cyst (LC Group), 5 cases (9.25%), cavity bigger than 15 mm, luteal tissue thicker than 3 mm.

- No corpus luteum (NCL Group), 4 cases (7.40%).

- Corpus luteum and follicle (CLF Group) bigger than 10 mm, 15 cases (27.77%).

- Small corpus luteum (SCL Group), less than 17 mm, 15 cases (27.77%).

Jaśkowski et al. (2021), reported a mean serum P4 concentration of 8.84 ng/ml, higher ($P < 0.0001$) for females with the cavitary Corpus Luteum (11.31 ng/ml) than for those with the homogenous (compact) Corpus Luteum (7.15 ng/ml). In our study, the situation was the opposite, the serum P4 concentrations were higher in the CL group (2.022 ng/dl) than in the HCL group (1.16 ng/dl). However, in our study, just in the case of Luteal cyst group (7.03 ng/dl) the values were similar to the values from Jaśkowski et al.(2022) study, in the other groups the values were significantly lower (1.16-2.022 ng/dl)(Table 2). Jaśkowski B.M. et al. (2022) reported that the presence of a cavitary Corpus Luteum in the recipient heifers did not negatively affect the potential of the CL to maintain pregnancy. On the contrary, the cavitary Corpus Luteum may give the embryo a better chance of surviving the time of pregnancy recognition and in consequence, may have a positive effect on pregnancy in heifers (Jaśkowski et al., 2021).

Table 2 P4 levels for each group

P4 levels	CL group	HCL Group	LCGroup	CLF Group	SCL Group
Sample 1 (ng/dl)	2.080	0.94	7.03	1.12	2.13
Sample 2 (ng/dl)	2.018	1.13	6.56	1.69	2.45
Sample 3 (ng/dl)	2.097	1.41	7.51	2.12	1.45
Average	2.022	1.16	7.03	1.64	2.01
Sd p=0.5	0.033941	0.19799	0.671751	0.304056	0.707107
SE p=0.5	0.04704	0.2744	0.931	0.4214	0.98

Since the embryo is transferred into the ipsilateral horn of the uterus, several studies have shown that a CL of at least 17 mm in its diameter (Yoshida et al., 2016) guarantees a satisfactory conception rate and maintenance of pregnancy, for lower pregnancy rates in heifers receiving fresh embryos if their progesterone (P4) level was below 1 ng/mL, and for previously frozen embryos when the P4 concentration was less than 3 ng/mL. The percent of SCL (less than 17 mm in diameter) in our study was 27.77, with the mean values of P4 of 2.01.

Thomas et al. (2021) reported that conception rates were similar for recipients with a central lacuna (62%, n = 245) and recipients with no central lacuna (66%, n = 448) (P = 0.10), and also states that no effect on conception rate was found with either the small (<50% of CL diameter) or large (>50% of CL diameter) central lacunae (P = 0.18). Spell et al. (2001) said that the best gauge of the suitability of a potential embryo transfer recipient is an observed estrus and a palpable corpus luteum, regardless of size or quality. Concentrations of progesterone at the time of embryo transfer were not predictive of pregnancy rates after embryo transfer.

Regarding the P4 levels, Nogueira et al. (2004) reported a decline in conception rates in the animals with the highest plasma progesterone concentrations, in our study the highest plasma progesterone concentrations were found in the LC group. Contrary, Dorneles Tortorella R. et al. (2013), reported a higher pregnancy rate in the cows with high blood progesterone levels.

CONCLUSIONS

Finally, due to the diversity of viewpoints on this subject, establishing suitable recipients in Embryo transfer programs remains a significant difficulty. The cystic corpus luteum and the small corpus luteum, appears to be a good choice for the Embryo transfer. Because there are no

published data on the presence of a follicle, more research should be done in this area. Even though the P4 concentrations are maximum in the case of a luteal cyst, Embryo Insemination in these circumstances is fraught with risk and should be avoided.

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