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Maternal and non-maternal factors associated with late embryonic and early fetal losses in dairy cows

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ABSTRACT

Defining factors associated with embryonic and/or fetal losses will be helpful in overcoming such problem, either by adjusting conditions or applying therapeutic approaches to high-risk cows. The objective of this study was to investigate the association between a number of maternal and non-maternal factors and the risk of late embryonic (LED) and early fetal death (EFD) in dairy herds. Additionally, we investigated the effect of treating pregnant cows either with GnRH on day 26 post-insemination, or GnRH on day 26 plus CIDR insertion between days 26 and 33 post-insemination, on the risk of LED/EFD. From 3826 pregnancies, diagnosed at day 30 post-insemination, 851 cows lost the pregnancy by day 70 post-insemination. A mixed-effects logistic model was constructed to assess the effect of cow breed, calving difficulty, postpartum problems, lactation number, days in milk, insemination number, actual 305-day milk production, temperature humidity index (THI) at insemination, estrus synchronization protocols, and other factors, on the risk of LED/EFD. Our findings indicated that Holstein X Brown Swiss crossbreed cows had a lower risk for LED/EFD than Holstein cows ($P < 0.05$). Cows that had postpartum problems, were inseminated for the first time, produced more milk, or were inseminated at THI $\geq$75, recorded higher risks of LED/EFD ($P < 0.05$). Calving difficulty, lactation number, and synchronization protocols were not found to be associated with LED/EFD. Moreover, treatment of the pregnant cows with GnRH on day 26 post-insemination plus CIDR insertion between days 26 and 33 post-insemination decreased the risk of LED/EFD. In conclusion, cows that had postpartum problems, were inseminated early postpartum, produced higher milk, and/or were inseminated at high THI, were under higher risk of LED/EFD. Treating such cows with GnRH on day 26 plus CIDR insertion between days 26 and 33 may decrease the possibility of the LED/EFD.

1. Introduction

Pregnancy loss is an important form of infertility that significantly affects the reproductive and economic performance of dairy herds [1]. Pregnant cows may lose their pregnancy at different stages, beginning from a few days after fertilization up to few days before the due date [1,2]. The majority of the pregnancy loss was recorded during the embryonic or early fetal stage [3]. Although prolonged inter-estrus interval may be considered as an indicator of early embryonic death, accurate diagnosis of this condition requires complicated procedures such as uterine lavage [4] or analysis of pregnancy-associated proteins [5]. With routine field application of ultrasonographic examination for early pregnancy diagnosis (around day 30 post-insemination), it becomes comparatively easy to study the extent of late embryonic (LED) or early fetal death (EFD). The LED/EFD is a common problem, especially in high lactating dairy cows [6], with a prevalence that varies from 7.2% to 29% [7,8].

Several maternal and non-maternal factors have been found to influence the embryonic or fetal death [9]. The maternal factors include cow body condition score (BCS) [10,11], milk production

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[10,12,13], days in milk (DIM) at insemination [12], calving to first insemination [13], the number of lymphocytic foci in the endometrium [14], postpartum problems (e.g. retained placenta, metritis, and chronic endometritis [15,16]), age of the dam at insemination, the number of pregnancies, the number of previous abortions, and the outcomes of previous pregnancies [17]. The non-maternal factors include the season of insemination [10,11], insemination sire [7], maternal sire [13], fetal anomalies or position of the fetus in relation to the corpora lutea (CL) [18], farm management [11], and estrus synchronization protocols [19,20].

The probability of pregnancy loss may be predicted either by estimating the serum concentrations of pregnancy-associated glycoproteins (PAGs) [5,21] or progesterone [12,22], or by designing a statistical model to define cows with a high probability of pregnancy loss [17]. If conditions associated with high risk of pregnancy loss are determined, it will be possible to reduce the incidence of pregnancy loss by correcting these conditions and/or applying a therapeutic approach to high-risk cows [23].

Previously therapeutic approaches include the application of an exogenous source of progesterone [24] and administration of hCG [25] or GnRH, either as a deslorelin implant [26] or gonadorelin [24]. The latter approach aims to maximize the internal source of progesterone either by formation of accessory CL or increasing the progesterone production rate from the main CL. Otherwise, it is expensive and impractical to treat all pregnant cows. However, estrus resynchronization before pregnancy diagnosis may be applied to reduce the inter-insemination interval [27]. The first treatment in the resynchronization protocol is either GnRH, progesterone, or both [27,28]. Resynchronization with GnRH at day 21 post-insemination do not affect the embryonic losses between days 28 and 42 [27]. However, resynchronization with CIDR between days 28 and 35 decreases the embryonic and fetal losses between days 28 and 90 [28].

We hypothesized that several maternal and non-maternal factors modulate the risk of LED/EFD through modification of progesterone production. Therefore, the objectives of the present study were twofold: (i) to investigate maternal and non-maternal factors associated with the risk of LED/EFD between days 30 and 70 post-insemination in dairy herd, and (ii) to assess the effect of treating pregnant cow either with GnRH on day 26 post-insemination, or GnRH on day 26 plus CIDR insertion between days 26 and 33 post-insemination, on the risk of LED/EFD.

2. Materials and methods

2.1. Cows and herd management

The data were generated at a private commercial dairy farm (Alkassem Farm, Cairo-Ismailia Road, Egypt) over the period from September 2011 to August 2015. This study was approved by the Committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine, Zagazig University, Egypt. The total number of cows enrolled in the current study was 1789, included 1570 Holstein, 76 Brown Swiss, and 143 crossbreed (Holstein x Brown Swiss). Cows were housed in free stall yards supplied with shelters and a cooling system, including fans and water sprinklers. Cows were fed *ad libitum* a total mixed ration (TMR) that was formulated according to the NRC [29] and adjusted to cover the cows' requirements according to their milk production. Cows had free access to water and were milked three times daily. The actual cumulative milk yield over the first 305 days was automatically recorded for individual cows.

Cows were examined twice for Brucellosis and once for tuberculosis per year. Animals were under a tight vaccination program against BVD, IBR, *Leptospira* spp., *Clostridia* spp., RFV, *Pasteurella*, Lumpy skin disease, Foot and mouth disease, and Ephemeral fever. Cows were supplied with pedometer systems as a tool for estrus detection. The data generated by the pedometers were analyzed with AfiFarm (Afimilk Ltd. Kibbutz Afikim, Israel). The daily temperature and humidity were obtained from the National Meteorology Station located at Cairo airport, about 10 km from the farm. The daily temperature humidity index (THI) was calculated as previously described [30].

$$THI = (1.8 * AT + 32) - ((0.55 - 0.0055 * RH) \times (1.8 * AT - 26))$$

where *AT* denotes the ambient temperature and *RH* is the relative humidity.

2.2. Reproductive strategy

2.2.1. Examination of postpartum cows

Calving difficulty was classified as easy birth (cows give birth without any interference), difficult (cows delivered with forced extraction procedures), and very difficult (cows delivered with mutation and forced extraction procedures). Postpartum cows were checked daily during the first 10 days after calving, and postpartum problems such as retained placenta and metritis were recorded and handled until recovery. Between days 25–30, postpartum cows were examined transrectally, ultrasonographically, and vaginally, when required to evaluate uterine involution and diagnose chronic endometritis and pyometra. Cows diagnosed with chronic endometritis were treated with intra-uterine infusion, with either 2 mg oxytetracycline (Delta Oxy, Delta Pharma, Egypt) or 500 mg cephapirin (Metricure, MSD, animal health). Cows diagnosed with pyometra were treated with 500 μg cloprostenol (Estrumate, MSD, Animal Health), followed by intrauterine infusion with oxytetracycline or cephapirin.

2.2.2. Estrus synchronization for the first insemination

Parturient cows were assigned to estrus synchronization protocols to get the first insemination at day 60 ± 3 postpartum. Presynch-ovsynch, Presynch-CIDR-ovsynch, or Double-ovsynch was initiated at day 30–35 postpartum, while Ovsynch or CIDR-ovsynch was initiated at day 50 postpartum. Cows in the Presynch protocol were synchronized with two doses of PGF2α (500 μg cloprostenol, Estrumate, MSD, Animal Health) on days 0 and 14. On day 28, cows were treated with GnRH (10 μg buserelin acetate, Receptal, MSD, Animal Health), followed by another dose of PGF2α on day 35. To induce ovulation, cows were treated with GnRH 56 h later and inseminated 16 h after the GnRH treatment. Cows in Presynch-CIDR were handled as described in the Presynch protocol, except for the insertion of CIDR (Eazi-Breed, Pfizer Animal Health) between days 28 and 35 (Fig. 1). In the Ovsynch protocol, cows were treated with GnRH on day 0, followed by PGF2α on day 7. Fifty-six h after PGF2α, cows were treated with GnRH and inseminated 16 h later. The CIDR-Ovsynch protocol is similar to Ovsynch, except for the insertion of CIDR between days 0 and 7. The Double Ovsynch protocol includes two Ovsynch protocols 7 days apart (Fig. 1).

2.2.3. Follow up and re-insemination

Inseminated cows that returned to estrus, up to day 30 post-insemination (based on data collected by pedometer and clinical examination), were re-inseminated. Non-returned cows were examined using an ultrasound scanner supplied with 7.5–10 MHZ linear-array transducer (My Lab One- Esaote, Italy) for pregnancy diagnosis at day 30 post-insemination. Cows showed CL in the ovary and embryo proper with heartbeats in the uterus, were diagnosed as pregnant. In the case of inability to detect the embryo or its heartbeats, the animal was classified as questionable and re-