BF-1423
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RESEARCH ARTICLE Indian Journal of Animal Research

Effect of Season on Quantity and Competence of Oocytes Recovered Transvaginally from Holstein Cows for *In vitro* Fertilization

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10.18805/IJAR.BF-1423

ABSTRACT

Background: Season of the year can affect the reproductive behavior in Holstein cows, altering the competition of the oocytes, reflecting a reduced production of embryos. The objective of this study was to evaluate the average of total oocytes, competition of oocytes and embryos in the *in vitro* production process at different season of the year in Holstein cows.

Methods: During the four seasons of the year, was performed on each of the oocyte donor cows (winter, n = 957; spring, n = 1571; summer, n = 1776; autumn, n = 1128), by *in vivo* transvaginal follicular aspiration technique after the collection were subjected to the embryos production *in vitro.*

Result: The highest number of total embryos were produced in winter and autumn, compared to spring and summer (3.76±0.16 and 3.54±0.18 vs. 2.73±0.11 and 2.45±0.10; respectively, P<0.05). During winter, a higher percentage of oocyte competition was observed, followed by autumn and spring and less competition shown in summer (26.03±0.39, 19.08±0.29, respectively, P<0.05). The quantity and competence of the oocytes collected and *in vitro* embryo production were drastically reduced during the hottest months of the year in this area of intense heat.

Key words: Competence of oocytes, Embryos, Holstein, *In vitro* fertilization, Season.

INTRODUCTION

It has been shown that the season of the year influences the reproductive behavior of dairy cows (Hansen, 2019) which is due to an alteration of the hypothalamic-pituitary-gonadal axis, especially in seasons with intense heat (Autukaité *et al.,* 2021; Souza-Cácares *et al.* 2019). In this sense, the heat stress (HS) decreases fertility in dairy cows causing a decrease in milk production (Khanday *et al.,* 2019; Sandip *et al.,* 2019; Mellado *et al.* 2013), increases the days open and the interval between calvings and as a consequence, increases the culling rate of cows, which entails a considerable economic loss (St-Pierre *et al.* 2003).

The high ambient temperatures negatively affect reproductive efficiency (Wolfenson and Roth, 2019), among them are the modification of the estrous behavior of the cow reflected in a poor expression of estrus, the follicular dynamics (Sammad *et al.* 2020), the decreased competition of oocytes and embryo quality (Hansen, 2019). In addition, thermal stress causes a low proportion of embryos that reached the blastocyst stage (Roth, 2021) and increases the number of apoptotic cells in follicles (De Aguiar *et al.* 2020) and in embryos produced *in vivo* and *in vitro* (Edwards *et al.* 2009).

Despite these studies, few studies have been done obtaining oocytes in different seasons of the year with the *in vivo* transvaginal follicular aspiration technique (OPU) and subsequent *in vitro* embryonic production (IVP) in lactating dairy cows. The objective of this study, was to evaluate the average of total oocytes using the OPU technique on the competition of oocytes and embryos in the *in vitro* production process at different season of the year in Holstein cows.

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How to cite this article: Guerrero-Gallego, H.Z., Calderon-Leyva, G., Angel-Garcia, O., Guillen-Muñoz, J.M., Leyva, C., Mellado, M., Pedroso, R., Pessoa, L.G., Esparza, C. and Morales, J.L. (2021). Effect of Season on Quantity and Competence of Oocytes Recovered Transvaginally from Holstein Cows for *In vitro* Fertilization. Indian Journal of Animal Research. DOI: 10.18805/IJAR.BF-1423.

Submitted: 11-08-2021 **Accepted:** 14-10-2021 **Online:** 27-11-2021

MATERIALS AND METHODS

All handling and maintenance of the animals used in this study were in accordance with the guide for the ethical use of animals for research (FASS, 2010) and national (NAM, 2002).

Location of the study area and climatic conditions

The study was carried out in various commercial dairy farms in the same area (ratio of 35 km^2) of northern México, located

at coordinates 25° 31′ N and 103° 13′ W, at an altitude of 1,100 meters above sea level. The climatic conditions in this semi-arid region are mean annual rainfall of 230 mm; average maximum temperature of 41.4°C during May and June and the lowest (-2°C) during December and January. The relative humidity ranges between 20 and 55% (INIFAP, 2017).

Experimental animals and groups

For the development of the experiment, 5432 high-yielding Holstein cows between two and three calvings were used, with 60 to 90 days in lactation, with a body condition of 3.5 on a scale of 1-5 (Bernabucci *et al.* 2005). The health of all cows was monitored from calving to oocyte collection and only cows that did not have reproductive disorders were selected according to gynecological examination. The feeding and balancing of the diet was according to the requirements described by the NRC (2001) for cows weighing 650 kg and daily milk production of 35 kg. During the four seasons of the year 2017, OPU was performed on each of the oocyte donor cows (winter, n = 957; spring, n = 1571; summer, n = 1776; autumn, n = 1128).

The OPU was performed *in vivo*, using ultrasound equipment (CHISON, Digital Ultrasound System, Model: 8300 VET. 5.0 MHz Sectorial) connected to a vaginal transducer coupled to a follicular aspiration guide with a 20 G × 2" cannula, which is joined by a follicular aspiration system equipped with a 50 ml conical tube for collection with a vacuum pump (Pionner Pro Pump, Pioneer Pro Pump Single -115v, Single Foot Pedal, PS 653, Canada), according to the technique described by Solís *et al.* (2012).

The cumulus-oocyte complex (COC) collected in each aspiration session were counted, morphologically evaluated and classified, taking as a reference the criteria proposed by De Loos *et al.* (1989) using grade I, II and III oocytes. After the evaluation, COC were subjected to the IVP process according to the technique described by Paula-Lopes and Hansen (2002), in the Reproductive Biotechnology laboratory of the Autonomous Agrarian University Antonio Narro, Laguna Unit. The embryos obtained were morphologically evaluated following the procedure of Bó and Mappletoft (2013).

The variables evaluated in this experiment were: average the oocytes recovered, total embryos produced derived from oocytes collected and percentage of oocyte competition. Developmental competence is defined as the oocyte's ability to mature and undergo fertilization and further embryonic development (Roth, 2017).

Statistical analysis

Data were evaluated using an ANOVA in a completely randomized design (PROC GLM of SAS; SAS Inst. Inc., Cary, NC, USA). In case of observing significant differences among seasons, the comparison of means was carried out with the PDIFF option of SAS. The differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Total oocytes recovered

Fig 1 shows the results of the total average of oocytes collected by OPU in Holstein cows during the four seasons of the year. The total production of oocytes among seasons differed ($P<0.05$). The highest number of oocytes was obtained in winter, followed by autumn ($P<0.05$). The lowest oocyte production was observed in spring and summer, without a not significant difference between them ($P>0.05$).

In this study, it was observed that the number of oocytes obtained per cow was lower in the seasons (spring and summer) with more intense heat, this probably was due to the effect of higher environmental temperature, which affect the number of oocytes (De Rensis *et al.* 2015), while in the wintertime a greater quantity of oocytes produced per cow was observed. This adverse effect of high ambient temperature on reproduction has been reported in various studies (Hansen, 2019; Wolfenson and Roth, 2019). Results in terms of oocytes obtained in this work and from previous studies may be due to the modification of the follicular dynamics of cows during high environmental heat, which alters follicular dominance and decreases the size of medium-sized follicles (Roth, 2021). Also, HS alters follicular development and compromises the development potential of oocytes (Paula-Lopes *et al.* 2012) and this, in turn, could affect the number of suitable follicles at the time of OPU. High environmental heat alters the hypothalamic-pituitary-gonadal axis, ovarian follicles in the different stages of development, these could modify the general pattern of follicular wave dynamics (Wolfenson and Roth, 2019) thus, affect the oocytes obtained from cows subjected to OPU.

***In vitro* embryo production**

Fig 2 shows the average number of total embryos derived from oocytes collected by OPU in Holstein cows during the four seasons of the year. The highest number of total embryos were produced in winter and autumn, compared to the spring and summer seasons ($P<0.05$).

Regarding embryo production, the results were consistent with the competition of oocytes, since fewer embryos were produced in the summer, followed by spring and there was a greater embryo produced in winter and autumn. These results may be explained by the findings of Walsh *et al.* (2011) who documented that the inability of embryonic development is due to the poor quality of the oocyte, which, as explained above, was found in the most intense hot seasons.

In various studies it has been proven that HS has unfavorable effects on the oocyte, these same effects being the ones that could lead to a reduction in embryo production. In this study, cleavage was not evaluated, however and according to Gendelman and Roth (2012), the rate of cleavage regardless of season is high when compared to fertilized oocytes that complete their development into blastocyst. It could be that this is independent of the fact

that the oocytes in the season of intense heat are fertilized and may even cleave, as the competition of the oocytes is affected as previously verified. Fertilized ovum may not have progressed in their development and probably for this reason

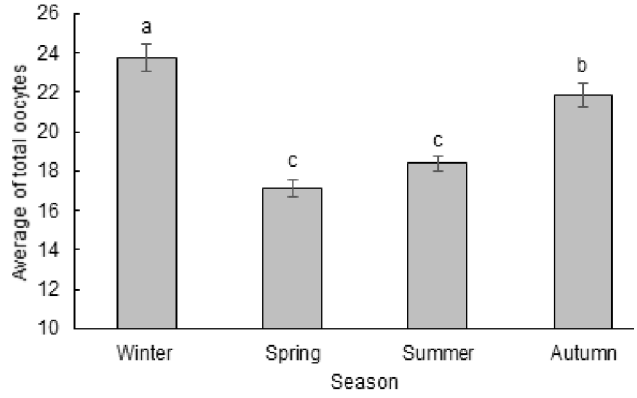

Fig 1: Total oocytes (mean ± SEM) collected per cow *via* OPU in Holstein cows (n = 5432) during the four seasons of the year. a, b, c = Different letters indicate statistical difference (P≤0.05).

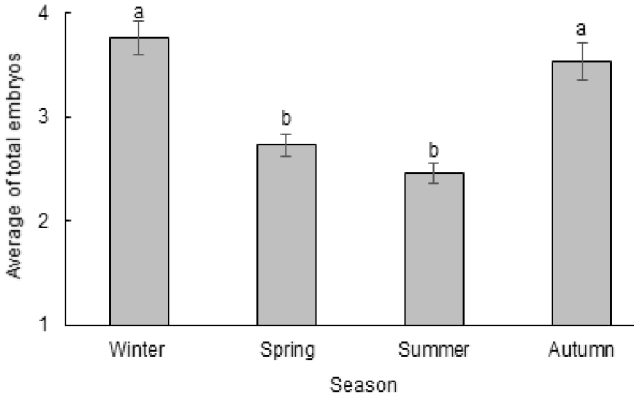

Fig 2: Total embryos (mean ± SEM) derived from oocytes collected per cow using the OPU technique in Holstein cows (n = 5432) during the four seasons of the year. a, b, = Different letters indicate statistical difference (P≤0.05).

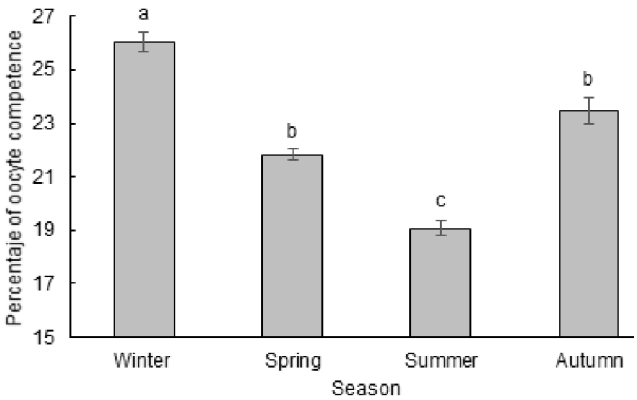

Fig 3: Percentage of competition of oocytes collected per cow using the OPU technique in Holstein cows (n = 5432) that developed into embryos (mean ± SEM) during the four seasons of the year. a, b, c = Different letters indicate statistical difference (P≤0.05).

embryonic production was lower. In another experiment, it was reported that heat stress during *in vitro* fertilization decreases fertilization success (Sakatani *et al.* 2015), which supports the explanation of the lower embryonic production in the seasons with intense heat compared with seasons with less severe heat (Payton *et al.* 2004).

Competition of oocytes

Fig 3 shows the percentage of competition of oocytes collected by OPU throughout the year that developed to embryos in Holstein cows. The percentage of oocyte competition through the times of the year significantly differed. That is, a higher (P<0.05) percentage of competition was observed during winter, followed by autumn and spring seasons and the poorest competition occurred during summer (P<0.05).

In this sense, the competence of oocytes is a complex process that involves the integral maturation of the oocyte and the acquisition of the capacity to achieve its development (Khanday *et al.,* 2019; Hansen, 2019). In the same way that was observed in the above-mentioned variables, the competition of the oocytes was greater in the temperate seasons compared to seasons with intense warm weather. Some reports indicate the effect of season on the development of oocytes (Silva *et al.* 2016; Gendelman and Roth, 2012) and, as in the present study, a decrease in their growth and maturation was reported.

Again, the results of the present study could also be supported by what was proposed by Wolfenson and Roth (2019), who mention that HS for prolonged periods could alter the follicular microenvironment, damaging the competence of the cell and for this reason, the oocytes would have less capacity to cleavage and become an embryo. These findings could explain the decrease in fertility of Holstein cows in hot months in the region where the present study was carried out (Mellado *et al.* 2013).

Oocytes collected from Holstein cows during the summer have reduced oocyte competition and this is expressed by delayed division and a low rate of embryo development to the blastocyst stage. This through the mechanism by which HS affects the competition of oocytes implies cellular and molecular damage, resulting in a failure in the maturation and fertilization of the oocytes (Roth, 2021).

CONCLUSION

The objective of this study was to evaluate the average total oocytes using the OPU technique on the competition of oocytes and embryos in the *in vitro* production process at different times of the year in Holstein cows. We conclude that the quantity, competition of oocytes and embryo production *in vitro* varied markedly according to the time of year in which oocytes were collected from Holstein cows in an area of intense heat, likewise, the time that had the greatest negative effect on the variables evaluated were summer compared to winter.

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