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Seasonal Influence on Oocyte Recovery Rate, Quality and *in vitro* Maturation in Cows

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ABSTRACT

Background: Recent breakthroughs *in vitro* maturation, fertilization and culture technology have allowed for an increase in the number of offspring generated from genetically better females, but this progress is still hampered by critical factors affecting oocyte yield and quality. This study aims to evaluate the effect of the seasons on the rate of maturation and recovery of oocytes.

Methods: The ovaries were brought from the slaughterhouse in Riyadh, Saudi Arabia, in a 0.9% NaCl saline solution and brought to the laboratory within 2-3 hours at a temperature of 25-35°C. Only the collected oocytes with two layers or more of cumulus cells (CCs) and uniform ooplasm were used. Five repetitions were made in each season of the year, followed by their ripening in the laboratory and a follow-up ripening after 24 hours. Observe the maturity rate after the stain and the expansion of cumulus cells and compare them with other seasons.

Result: The mean maturation rates of MII and MI oocytes were not significantly different between all seasons. Additionally, there were no significant changes in the cumulus-oocyte complex before maturation in all seasons. However, after maturation, a highly significant difference (P<0.001) was found between the spring season before and after maturation (156.31±17.68 mm) and the rest of the seasons (summer; 169.89±19.96 mm; autumn; 176.66±20.14 mm; and winter; 188.84±24.50 mm). In order to improve *in vitro* maturation for following investigations, such as *in vitro* fertilization or cloning and *in vitro* embryo development, the ideal season should be chosen for collecting oocytes.

Keyword: *In vitro* maturation, Oocyte, Ovary, Seasonality.

INTRODUCTION

Animals can be used for postmortem follicular aspiration, ovariectomy, or ultrasound-guided follicular aspiration to obtain oocytes for *in vitro* production. In ovaries obtained from abattoirs, oocytes are aspirated from a variety of antral follicles. These antral follicles have a size range of 2 to 8 mm. As well as from dominant and subordinate follicles within each wave, these follicles originate from both ovulatory and non-ovulatory follicular waves. Oocytes from the larger, more dominant follicles will undergo a process called “pre-maturation” or “capacitation,” which boosts their capacity for later maturation, fertilization and maintenance of embryonic development. This process is essential for reproductive success, as it enables the oocytes to move through meiotic divisions and have the correct cytoplasmic components for successful fertilization. *Bos taurus* females who have not been stimulated by FSH can produce four to five acceptable (grades 1 and 2) oocytes each donor session when using follicle aspiration, but Holstein females that have been stimulated can produce up to twenty oocytes per donor session (Hyttel *et al*., 1997; Bols *et al*., 2005; de Loos *et al*.,1989; Hasler, 1998; Vieira *et al*., 2016). Follicular aspiration can be performed on donors once to twice a week, with or without stimulation (Chaubal *et al*., 2007).

The growing gamete goes through both nuclear and cytoplasmic alterations throughout oocyte maturation. The transition during meiosis from the prophase of the first meiotic division to the metaphase of the second meiotic division (MII) at the time of ovulation is known as nuclear oocyte maturation. Along with nuclear maturation, the oocyte undergoes cytoplasmic oocyte maturation, which involves changes to its organelles, proteins and transcripts. Meiosis is halted at MII until fertilization, at which point it is resumed and the second polar body segregation is accomplished (Hyttel *et al*., 1999; Sirard, 2001). Oocytes typically reach MII nuclear maturity under *in vitro* conditions within 20-24 hours, at which point they are prepared for fertilization (Ammari *et al*., 2022).

In bovine, a non-seasonal species, the blastocyst rate was lowest when oocytes were collected in the summer, likely as a result of the hot weather and poorer feed quality in IVP systems. The cleavage and morulae development rates were also lowest when oocytes were collected in the autumn compared to the other three seasons (Gupta and

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Aznar, 2016). Curiously, in a subtropical area, the ambient temperature substantially impacted women's pregnancy rates following *in vitro* fertilization (IVF) (Zhao *et al*., 2019). Studies investigating the effect of temperature found that buffalo and sheep produced superior outcomes throughout the winter (Abdoon *et al*., 2014; Ahmad *et al*., 2019).

The majority of research teams have noted yearly fluctuations in embryo output in seasonal breeders. For instance, more oocytes of greater quality and quantity were extracted from Zandi ewes during the breeding season, enabling them to produce more blastocysts (Davashi *et al*., 2014). Generally, compared to anestrus, the breeding season yields greater *in vitro* production rates (Mara *et al*., 2013). However, the cleavage and blastocyst rates in prepubertal goats were considerably greater in the nonbreeding season than in the breeding season (41 N latitude) following *in vitro* fertilization (Catala *et al*., 2018), The objective of this study is to determine how the altering of the seasons influences the rate of oocyte maturation and recovery.

MATERIALS AND METHODS

Unless otherwise mediums all chemicals and mediums utilized in the current study were brought from Sigma-Aldrich Co. (St. Louis, MO, USA) and Caisson Laboratories (Smithfield, Utah, USA). Our study was taken the approval by the Animal Ethics Committee of King Saud University (KSU), KSA. And this experiment was done at the embryonic studies laboratory, Department of Zoology, College of Science, KSU, KSA.

The city of Riyadh sits directly in the center of the Saudi Arabian kingdom. It covers the area between 24°38 N and 46°43 E in longitude. It has a dry and arid climate, with daytime highs of 35 to 43 degrees Celsius and lows of 20 to 25 degrees Celsius at night throughout the summer. The winter months saw a significant drop in temperature, sometimes dipping below freezing. Around 600 meters above sea level, Riyadh City boasts a number of valleys and many sand dunes.

The ovaries were transported in a saline solution containing 0.9% sodium chloride from the slaughterhouse in Riyadh, Saudi Arabia, to the laboratory within two to three hours at a temperature that was maintained between 25 and 35°C. Aspiration using a needle of 20 gauge carrying oocyte collection media TCM199 Hanks2 salts medium + 0.14 mg/ml heparin sodium salt+4 mg/ml bovine serum albumin (BSA) was used to remove the oocytes from the ovary and collect them. Oocytes were only employed if they had at least two layers of cumulus cells (CCs) and had consistent ooplasm. oocytes were grown in TCM199 Earle's salts supplemented with 10% fetal bovine serum (FBS), 0.5 mM sodium pyruvate, 0.02 IU/ml FSH, 0.023 IU/ml LH, 1 g/ml Estradiol 17β, 100 mM cysteamine and 50 g/ml gentamycin. Oocytes were grown in a 24-hour incubation at 38.5°C, 5% CO_2 and high humidity (Al-Mutary *et al*., 2019; Ammari *et al*., 2022).

Based on our previous studies, we looked at the morphometric parameters of the oocytes (Ammari *et al*., 2022). A camera and the software that came with the microscope were used to take the images (Leica Application Suite, Version 4.0), Image 1.50i was used to assess the morphometric properties of the recorded pictures (NIH, USA), The program used the scale bar as an arbitrary scale to analyze individual pixels in the same oocyte picture; the results were saved. Morphometric characteristics can be used to describe both the immature and mature forms of the Cumulus oocyte complex.

At the end of the oocyte maturation phase, the cumulus cells were gently pipetted out of the mature oocytes. After being stained with 1% Aceto-Orcein (A-O) and rinsed in a solution of distilled water, glycerol and acetic acid (3:1:1), the sample was fixed in Acetic Acid + Ethanol (A/E) (1:3), put on a glass slide and overlaid. The subsequent steps involved recording the various stages of meiosis (Zabihi *et al*., 2019).

RESULTS AND DISCUSSION

Regarding the effect of the season on the maturation rate (MII) of *in vitro* matured cow oocytes with the MII trait, the results in Table 1 showed that there was no significant difference in the mean value of oocytes in all four seasons (spring, summer, fall and winter). Regarding the effect of seasonality on ovary and oocyte recovery rates, the results in Table 2 showed a significant difference between the mean value of the number of ovaries in the winter season (55±3.16) whereas in other seasons there were no significant differences. Also, there are no significant differences in the number of recovered oocytes across all seasons.

The complex revealed no significant diameter changes before the cumulus oocytes' maturity (Spring: 46.82±6.65 mm; Summer; 64.15±17.81 mm; Autumn 52.38±10.87 mm; 60.72±17.11 mm). However, after maturation, a significant difference was found in the diameter between the three seasons (summer, fall and winter) and a highly significant difference ($P<0.001$) was found in the diameter in the spring season compared to the rest of the seasons Table 3.

The optimal time of year to harvest oocytes from the ovaries will have an effect on the subsequent processes of

Table 1: Effect of the season in maturation rate (MII) of *in vitro* matured cow oocytes.

Maturation rate (MII Mean±SEM)	Spring	Summer	Fall	Winter	P-value
MII	115±10.12	133.60±18.74	85±17.18	116.20±12.87	0.19
MI	34.60±7.14	54.60±17.56	42±20.03	59.60±16.58	0.68

Table 2: Effect of seasonality on ovary and oocytes recovery rate.

Ovary and oocytes recovery rate	Spring	Summer	Fall	Winter	P-value
Ovary	34.20^{b}±5.62	39.20^{b}±1.85	40^{b}±5.24	55^{a}±3.16	0.02
Oocyte	168±18	193.20±29.04	127.20±36.67	177.40±27.14	0.43

Table 3: Effect of seasonality on expansion rate in cumulus oocyte complex.

The expansion rate in the cumulus oocyte complex through maturation	Spring	Summer	Autumn	Winter
Cumulus oocyte complex before maturation (mm±SEM)	46.82^{b}+6.65	64.15^{b}+17.81	52.38^{b}+10.87	60.72^{b}+17.11
Cumulus oocyte complex after maturation (mm±SEM)	156.31^{a}+17.68	169.89^{a}+19.96	176.66^{a}+36.14	188.84^{a}+24.50
P-value	<.0001	0.002	0.006	0.001

in vitro maturation, *in vitro* fertilization or cloning and *in vitro* embryo culture, as well as improve the quality of embryo development. This is because the optimal time of year to harvest oocytes from ovaries varies depending on the individual's reproductive history. Several investigations have concluded that there is no effect (de Wit *et al*., 2000; Sungulle, 2008). The higher level of transcriptional activity in oocytes with a larger volume suggests that they have reached their full meiotic competence. This is in contrast to oocytes with a smaller volume, which is still in development (Fair *et al*., 1995).

This runs counter to the findings of a number of studies, which indicated that the pace of maturation changed depending on the season. It's possible that the different geographical locations have anything to do with that (Zidan *et al*., 2022; Maia *et al*., 2017; Zoheir *et al*., 2007; Rutledge *et al*., 1999). It was discovered that seasonality did not play a significant role, but rather that the effect of the month reflected natural variation from one month to the next (Rivera *et al*., 2000).

In addition, oocytes with 110-120 µm and 120 µm diameters achieved MII at similar rates (Fair *et al*., 1995) Oocytes attained meiotic competence at a diameter of 115 µm and full developmental capability at a diameter of at least 120 µm. Oocytes from bovine females with an inner zonal diameter of less than 95 µm are unable to resume meiosis *in vitro*; this includes a sizable percentage of bovine oocytes (Otoi *et al*., 1997).

The majority of oocytes harvested from the ovary for *in vitro* maturation come from immature follicles that are at least a few days away from being considered mature enough to release an egg. Even though most of these oocytes get to the point where their nuclei are ready, only a small number of them turn into blastocysts.

Meiotic progression and cumulus cell growth rate were used to assess the success of *in vitro* maturation. Oocyte maturation induction depends on the successful conclusion and integration of a number of important processes related to both the nuclear and cytoplasmic components of maturation (Moor *et al*.,1998). Meiotic development to metaphase II (MII) can be accelerated using luteinizing hormones, which improved the original *in vitro* maturation procedure (de Oliveira *et al*., 2020).

CONCLUSION

In order to improve *in vitro* maturation for enhanced assisted reproduction technologies, such as *in vitro* fertilization or cloning and *in vitro* embryo development, the optimal time of year to collect oocytes should be selected. This is done to maximize *in vitro* maturation.

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