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The Potential Impacts of Selecting Viable Oocytes on Further Embryonic Development in Mammals: A Review

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ABSTRACT

Selecting viable fully grown germinal and matured oocytes were positively effect on further embryonic development and live birth. The use of feed and hormonal supplementation and biotechnologies to enhance oocyte competence was evaluated. In addition, the oocytes were selected based on ovarian follicular stage, follicles and oocytes morphology, follicular fluid characters and gene expression. Changes were obtained in oocyte maturation, fertilization and further embryo cleavage and development over selecting viable oocytes. The larger follicle diameter, the translucent follicle and the higher blood supply the higher developmental competence of resulting oocytes. The higher surrounding cumulus cells and homogeneity of cytoplasm the higher developmental competence of oocytes. In addition, oocytes obtained during luteal stage compared to those obtained during follicular phase were highly or comparably developed to embryos. Furthermore, follicular fluid characters and gene expression might predict the embryonic developmental competence of oocytes in addition to their viable staining with brilliant cresyl blue. In conclusion, the characters of ovarian follicles, oocytes, follicular fluid and gene expression have significant implications on further oocyte developmental competence in different mammalian species.

Key words: Embryos development, Follicles, Follicular fluid, Gene expression, Oocytes morphology.

Significant strides of progresses is achieved in selecting the higher developmental competence oocytes through the characters of ovarian follicles, oocytes morphology, follicular fluid and gene expression (Mohammed *et al*., 2005; Mlyczyńska *et al*., 2023). It has been known that several factors affect the ovarian follicle diameters and the contents of oocytes and follicular fluid parameters (Contreras-Solís *et al*., 2021; Castro-Modesto *et al*., 2022; Mohammed *et al*., 2020, 2024a,b,c,d; Cañón-Beltrán *et al*., 2024).

Oocyte developmental competence is gradually acquired during the processes of follicle growth and maturation (Al Zeidi *et al*., 2022a,b; Mohammed *et al*., 2022; Aljubran *et al*., 2023). Modification of follicle environment through the use of feed and hormonal supplementation and biotechnologies to enhance oocyte competence was confirmed in several studies (Gordon 2003; Costa *et al*., 2023; Mohammed *et al*., 2024a,b,c). Several studies concerning the nutrition and feed additives, hormonal supplementation indicated that they were pivotal factors in determining the ovarian follicle development and oocyte quality that resulted in varied reproductive performances in different animal species and human (Mohammed 2018, 2019; Mohammed *et al*., 2022; 2024a,b,c,d; Ali *et al*., 2021; Al-Mufarji *et al*., 2022a,b; Mohammed and Al-Suwaiegh 2023; Al-Mafurji *et al*., 2023). Bragança *et al.* (2020) found that progestogen administered to sheep did not affect the pattern of ovarian first wave follicle populations and the resulting oocyte quality during ovarian stimulation with FSH. Primordial follicle activation and survival during *in vitro* culture of bovine ovarian tissue to control oxidative stress upon melatonin treatment was confirmed through different mechanisms (Silva *et al*., 2024). Lucia Dos Santos Silva *et al.* (2023) reviewed the role of antioxidants in prevention follicular damage caused by oxidative stress in the *in vitro* culture of preantral follicles. Therefore, our objectives were to highlight key aspects of nutrition, feed supplements and hormones on follicle and oocyte development in addition to selecting viable oocytes according to follicular and luteal cycle stages, morphology of follicles and oocytes, follicular fluid characters and gene expression (Fig 1-3).

The current study was carried out according to the procedure approved by Deanship of Scientific Research, King Faisal University, Saudi Arabi from September to March 2024. The earlier reports concerning the effects of nutrition, feed supplements and hormones in addition to follicular and luteal stages on oocytes developmental competence were utilized. In addition, the research papers concerning follicle and oocyte morphology, follicular fluid and gene

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expression in addition to assisted reproductive techniques were reviewed and discussed for selecting viable oocytes, increasing outcomes of reproduction and treatment of infertility. Therefore, our targets are to highlight key aspects of nutrition, feed supplements and hormones on follicle and oocyte development followed by selecting viable oocytes based on follicular and luteal estrous stages, morphology of follicles and oocytes, follicular fluid characters and gene expression.

Most reproduction management technologies and interventions are centered on the ovarian follicle development and the contents of oocytes and follicular fluid.

Nutrition and supplements

A plethora of molecules modified by the nutritional intake as amino acids, pyruvate, fatty acids, inorganic ions, second messengers, cytokines, growth factors effect on ovarian follicle development, oocyte quality, maturation and further embryonic development and live birth in different mammalian species (Mohammed 2018, Mohammed *et al*., 2020, 2024a,c; Hye *et al*., 2020; Thuy Van *et al*., 2020; Fabozzi *et al*., 2021; Costa *et al*., 2023; Garza *et al*., 2023; Silva *et al*., 2023; Liu *et al*., 2024).

Protein played a crucial role in both follicle growth and development and the quality of resulting oocytes (Dupont *et al*., 2014; Gao 2020; Rubio *et al*., 2021; Machado *et al*., 2020). Amino acids served as building blocks for protein synthesis that were essential for follicles' growth and development, granulosa cell proliferation and oocyte quality. Protein in the oocyte was used for synthesis of RNA and to store materials for sustaining future embryo development until maternal zygotic transition or genome activation, which occured in most of mammalian species between the two- to the eight-cell stages (Li *et al*., 2013). In addition, certain proteins function as antioxidants, protecting follicles and oocytes from oxidative stress, which damage DNA and impair their development. On the other hand, unbalanced protein diet resulted in alterations of structure, function and biogenesis with increased levels of reactive oxygen species (Schutt *et al*., 2019).

Carbohydrates were essential sources of energy for the body function including the reproductive system (Dupont *et al*., 2014; Fabozzi *et al*., 2021). Ovarian follicular growth and development and oocytes require a readily available supply of energy through carbohydrates. Report of Mohammed *et al.* (2021) indicated accelerated and higher development of ovarian follicle with propylene glycol supplementation. On the other hand, the potential negative effects of high carbohydrate intake over increased oxidative stress and hormonal imbalances could be damaging to DNA and cellular components within the follicles and oocytes.

There are molecular mechanisms of functional fatty acids affecting follicular development, granulosa cell steroidogenesis and oocyte maturation (Zeng *et al*., 2023). There was a strong relationship between fat supplementation and follicle size and oocyte quality (Khatir *et al*., 2007; Yang *et al*., 2016; Labrecque *et al*., 2016). The unsaturated fatty acids yielded positive effects on reproductive performance but the saturated fatty acids were linked to negative outcomes as decreased follicle number and quality (Zeng *et al*., 2023). On the other hand, the negative effects of high-fat diets included increased inflammation and oxidative stress, hormonal imbalances and decreased blood flow (Dupont *et al*., 2014). Collectively, abnormal dietary consumption of carbohydrates, fatty acids, proteins, vitamins and minerals, had detrimental effects on ovarian functions, affecting not only the oocyte quality but also the quality of embryos and implantation of a healthy embryo (Noli *et al*., 2020).

Feed supplements

Effects of nutritive and non-nutritive feed supplements on reproductive performances in mammals has been discussed (Mohammed *et al*., 2024a,b). The nutritive and non-nutritive feed supplements change rumen microorganism, feed utilization, body weight gain, blood and plasma profiles (Mohammed *et al*., 2024a,b,c). Such improvements over feed supplement/s lead to improvement in ovarian follicle development and the resulting oocyte quality, embryos and newborns in addition to amelioration of reproductive dysfunctions (Al Mafurji *et al*., 2022a,b).

The essential macronutrient and antioxidant micronutrient improved the reproductive performances through significant increase in concentrations of reproductive hormones, numbers and sizes of ovarian follicles in addition to increase in oocyte and embryo quality (Senosy *et al*., 2017, 2018, 2019; Mohammed 2018; Ali *et al*., 2021). In addition, green algae as a source of beta-carotene was supplemented to support oocyte and embryo quality in goats (Silva *et al*., 2023). The microalgae *Chlorella* did not enhance the quality of oocytes whereas it improved the quality of embryos and stimulated their mitochondrial function. Previous reports on *Dunallela saliena* resulted in improvement in levels of reproductive hormones, oocytes and embryo development and (Senosy *et al*., 2017; Mohammed 2018; Ali *et al*., 2021). Therefore, the continuous research in nutritive and non-nutritive feed supplements was necessitated for both animals and humans. Therefore, the future prospective studies to find beneficial new feed additives was an emerging approach for improving ovarian structural development and the resulting quality of oocytes and embryos (Mohammed *et al*., 2024a,b,c,d).

Hormonal supplementation

Hormonal supplementation had various effects on ovarian follicle development, oocyte quality and embryo development, depending on the specific hormone, dosage and body health conditionin mammalian species (Gordon 2003; Mohammed and Attaai, 2011). The important hormones related to ovarian follicular growth and maturation were follicle stimulating hormone, luteinizing hormone (LH), estrogen and progesterone. Follicle stimulating hormone (FSH) was used routinely in protocols of assisted reproductive techniques but excess FSH could lead to overstimulation and complications. Supplementation of LH and estrogen in specific contexts could support ovarian follicle and oocyte maturation but required careful management due to potential adverse effects. It was well-known that the physiological effects of progesterone during the pre-implantation stages of mammalian embryos was mediated by progesterone receptors and their gene expression. Although progesterone could not improve *in vitro* maturation rates, culture media supplemented with progesterone significantly improved mouse embryo development (Andersen 1990; Mendoza *et al*., 2002; Gordon 2003; Salehnia and Zavareh 2013; Flores-Herrera *et al*., 2008). Growth hormone injection improved follicles and oocytes development in prepubertal lambs (Liu *et al*., 2023). Collectively, hormonal supplementation could be double-

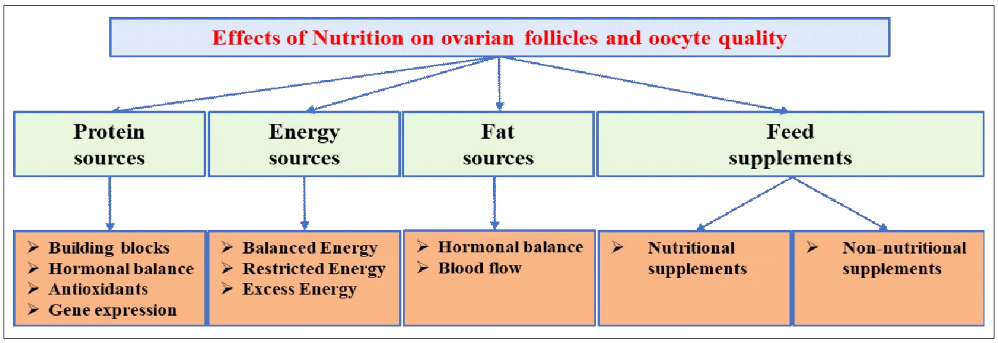

Fig 1: Effects of nutrition on ovarian follicle development and oocyte quality.

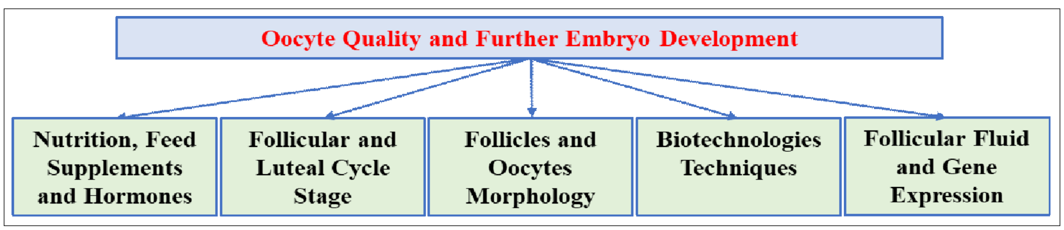

Fig 2: Factors responsible for selection of viable oocytes.

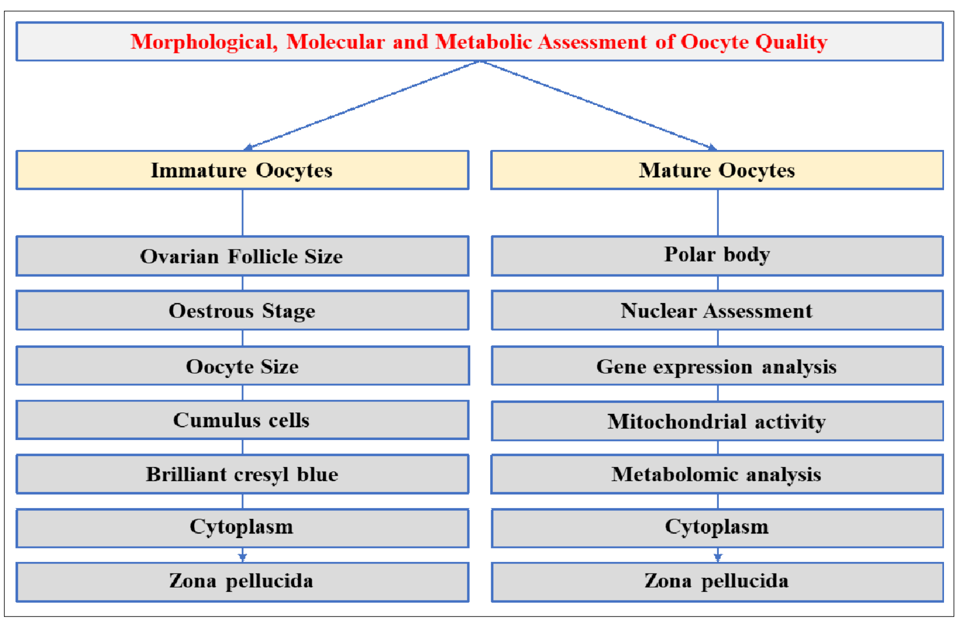

Fig 3: Morphological, molecular and metabolic assessment of oocyte quality.

edgedto either give a positive or negative effect depending on several factors.

Follicular and luteal stage of the cycle

Ovarian follicles develop through two processes called *initial* and *cyclic* recruitments (McGee and Hsueh, 2000), which describe recruitment of dormant primordial follicles continuously into the growing follicle and antral follicles, respectively. After puberty, multiple antral ovarian follicular waves (2, 3 and 4 waves) were reported in several studies (Gordon 2003; Baerwald and Pierson, 2020) during ruminant estrous or human menstrual cycles. It was estimated for bovine early antral follicles to take about 40 days to progress to preovulatory follicles.

The estrous cycle was divided to follicular and luteal phases (Gordon, 2003). Ovarian follicular waves or follicular and luteal stages were factors affecting the quality of oocytes, oocyte maturation and resulting embryo development (Mohammed *et al*., 2012, 2022). Some studies suggested that the estrous stage with its hormonal fluctuations can potentially influence oocyte quality in different mammalian species (Gordon, 2003; Majumdar *et al*., 2023; Dastjerdi *et al*., 2024). Some studies suggest higher oocyte quality in early follicular phase due to more favorable hormonal environment. Some other studies suggest negative impact of the corpus luteum on oocyte quality, particularly on smaller follicles.

Evidence concerning the importance of progesterone support during ovum pick-up and *in vitro* embryo production (OPU-IVP) was ascribed in studies related to transvaginal follicular aspiration in cattle and improvement of *in vitro* embryo production (Simmons *et al*., 2023). For treatment of fertility disorders, comparison of abnormalities during follicular phase *in vitro* maturation of oocytes compared with luteal phase *in vitro* maturation in women was investigated (Hatirnaz *et al*., 2023). The results indicated differences in maturation and fertilization rates, quality of embryos or pregnancy outcomes between follicular phase and luteal phase in women with oocyte maturation abnormalities. Additionally, Suñol *et al*., (2023) compared follicular phase stimulation versus luteal phase stimulation in suboptimal women responders. The oocyte yield did not increase in follicular-phase stimulation compared to luteal-phase stimulation. De Wit *et al*., (2000) also found that the stage of oestrous cycle had no effect on the distribution of the cumulus-oocyte complexes (COC) over the grades of oocytes qualities or on the developmental capacity of COC. In human, stimulation of luteal phase in the same menstrual cycle was an effective strategy to rescue poor responders with follicular phase stimulation (Majumdar *et al*., 2023; Dastjerdi *et al*., 2024).

Morphology of follicles and quality of oocytes

The ovarian follicles are categorized to different groups including primordial follicles to preantral follicles and antral ovulatory follicles (Gordon 2003) (Fig 4-5). Healthy antral follicles larger than 3 mm contain competent oocytes that are competent to develop into preimplantation and post implantation embryos (Mohammed *et al*., 2005). The sizes of ovarian follicles were different among mammalian species. They are categorized into small (<3 mm), medium (3-5 mm) and large follicles (>5 mm) in small ruminant species (Senosy *et al*., 2017; Al Mufarji *et al*., 2023). Sizes of ovulatory follicles naturally resulted in multiple ovulations and influenced on the development of corpus luteum and fertility in cattle (Echternkamp *et al*., 2009).

The effect of follicle size on oocyte quality was a complex issue and generally a positive correlation was noted between follicle size and oocyte quality. Large ovulatory follicles (≥16 mm) are more likely to yield mature oocytes that are capable of normal fertilization and development into high-quality embryos compared to small follicles (<13 mm) which produced immature oocytes with lower fertilization and cleavage rates in large ruminant species and humans as well (Kahraman *et al*., 2017). However, the relationship between follicle size and oocyte quality was not always clear and there could be exceptions where the age, follicular waves and health conditions, could

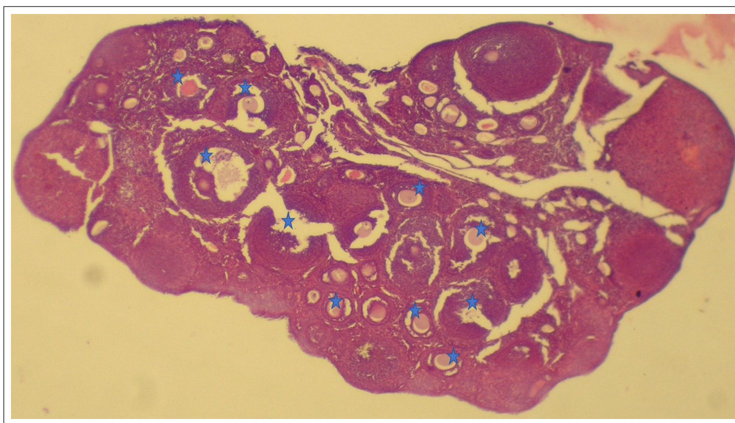

Fig 4: Mouse ovary containing preantral and antral follicles.

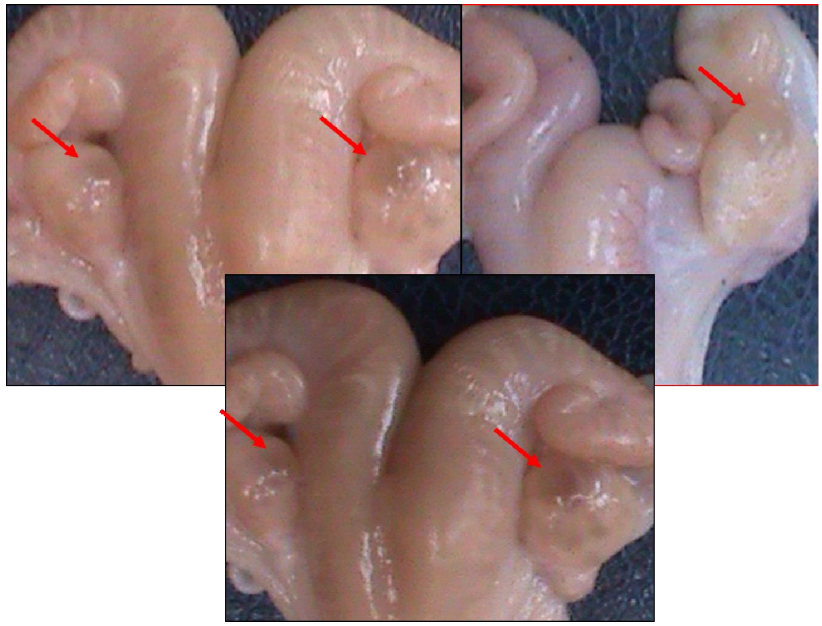

Fig 5: Translucent ovarian follicles in sheep ovaries.

also play a role in oocyte quality. Therefore, more research was needed to fully understand the complex interplay between follicle size and oocyte quality. Studies have shown that oocytes from larger follicles have higher fertilization rates and better competence for embryonic development (Mohammed unpublished report).

Follicular fluid characters

The correlation between follicular fluid characteristics and oocyte quality was complex and interesting field of research, with promising potential for improving assisted reproductive technologies (Mohammed *et al*., 2005, 2020). Follicular fluid (FF) surrounds the oocyte within the ovarian follicle and provides a crucial microenvironment for growth and development of the oocyte. Analyzing the chemical composition of FF offered insights into the ovarian follicle health and viability of the resulting oocyte (Mohammed *et al*., 2005; 2019a,b, 2020). Several studies had identified positive correlations between specific molecules in follicular fluid and oocyte quality such as insulin-like growth factors (IGFs) and their binding proteins (IGFBPs), which were often associated with higher maturation and fertilization rates of oocytes (Mohammed *et al*., 2019a). In addition, the higher levels of total antioxidant capacity (TAC) in follicular fluid seemed to indicate the presence of high quality oocytes in mature follicles. Furthermore, metabolites and certain cytokines have also shown promising connections to oocyte quality (Mohammed *et al.* 2019a,b; 2020).

Follicular fluid composition changed during the estrous cycle due to follicular growth and development and nutritional level as well (Mohammed *et al*., 2012). Other components still need further studies to define the changes at cellular level (Mohammed *et al*., 2019b). Finally, the research on the correlation between follicular fluid characteristics and oocyte quality was promising but still evolving due to differences in experimental design, animal species and the analytical methods used (Mohammed *et al*., 2020).

Gene expression

Complex relationship was noted between gene expression and oocyte quality. Gene expression in oocytes was influenced by intrinsic and extrinsic factors (Jentoft *et al*., 2023). The intrinsic factors include genetic encoding, age of female and epigenetic modifications. The extrinsic factors include follicular environment, maternal health, environmental factors and *in vitro* culture conditions during assisted reproductive techniques.

Functional molecules were produced through gene expression at different stages of oocyte development that played crucial roles in various processes (Maside *et al*., 2021). Gene expression regulated various aspects of oocytes including zona pellucida formation, spindle apparatus development, enzymes and molecules involved in energy production and utilization, molecules involved in the communication of oocyte with the surrounding cells (Wassarman and Litscher, 2021). For example, the zona pellucida genes encode sperm receptors, which were required for fertilization (Evsikov *et al*., 2009). The nutritional factors such as protein or lipids helped in regulation of gene expression in developing follicles and oocytes, influencing various processes crucial for successful development and maturation (Schutt *et al*., 2019; Sharma *et al*., 2020; Alves *et al*., 2021).

CONCLUSION

Nutritive and non-nutritive feed additives/supplements and hormonal injection are positively or negatively effect on ovarian follicle development and the resulting oocytes. Selecting viable oocytes was necessitated for assisted reproductive technology, which could be according to follicular and luteal phases of the estrous cycle, morphology of follicles and oocytes, follicular fluid characters and gene expression. Therefore, the future prospective studies to find newer methods for selection of viable oocytes was a continuous approach for improving reproductive performances.

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Conflict of interest

There is no conflict of interest for authors to declare.

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