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Original Paper

Effect of Ovarian Follicle Size on the Follicular Fluid's Hormones in Local Iraqi Bovine

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Bovine reproductive physiology is a complex process that is closely linked to the cattle industry's effective production. Follicle diameter and the hormonal content of follicular fluid play an important role in determining ovum quality, overall embryo production, and bovine fertility. This study investigates the hormonal profile of follicular fluid in ovarian follicles of varying sizes in Iraqi cows. A total of 22 samples were collected from sexually mature, disease-free, and non-pregnant cows over a two-month period (July and August). Three of the follicle groups were classified: (2–5 mm), (6–9 mm), and (10–15 mm). Following visual measurement, follicular fluid was extracted, and concentrations of progesterone, estrogen, testosterone, and thyroid-stimulating hormone (TSH) were analyzed. The results showed a significant difference (p <0.0001) in progesterone levels 6–9 mm. Also, estrogen concentration was higher in 10–15 mm compared to the other groups. Testosterone concentration was higher in follicles (2–5 mm) compared to follicles (10–15 mm and 6–9 mm), respectively. Thyroid-stimulating hormone was higher in 2–5 mm compared to 10–15 mm follicles. The current outcomes participate to the thoughtful of known mechanisms of hormonal function in ovarian follicle growth in bovine species, showing that progesterone, estrogen, testosterone, and TSH are critical factors in these mechanisms.

Keywords: local cattle, follicle development, estrogen, progesterone, testosterone

1 Introduction

Bovine reproductive physiology is a complex process that is closely linked to the cattle industry's effective production. Embryo production is a revolutionary assisted reproductive technique used for the production of superior offspring, which has spread worldwide; however, exploitation of embryo production techniques has faced challenges in the past decades (Mikkola et al., 2019). Embryo production *in vitro* involves pre-communication between the cattle follicle and the oocyte, which is vital for the oocyte's developmental competency to gain a successful pregnancy (Sirard & Blondin, 1996). Follicle diameter and follicular fluid content vary during the estrous cycle, which has a crucial character in determining ovum quality and overall embryo production, as well as bovine fertility (Thompson et al., 2007). There are two main follicle categories according to their size, e.g., large follicles greater than 10 mm and small follicles less than 5 mm. Naturally, a bigger follicle is joint with greater oocyte quality, which is important for embryo development and *in vitro* fertilization (IVF) (Sarwar et al., 2020).

Recently, hormonal content in the follicular fluid (FF) has turned out to be more effective for oocyte quality and IVF. Hormones, e.g., Progesterone, Estrogen, Testosterone, Insulin-like growth factor, and Inhibin, are also found in FF (Goodman, 2009). Estrogen is important for follicular development and maturation, inducing the estrous cycle and ovulation (Perry et al., 2023). Previous work by Kruip and Dielemon concluded that follicle selection can be determined by the concentrations of steroid hormones in the FF, which might be a possible process to predict ovum quality according to different follicle diameters (Kruip & Dieleman, 1985). Another study suggests that both progesterone and estradiol levels in the FF are linked to follicle size and oocyte maturation in beef cattle (Read et al., 2022). In addition to that, Progesterone is produced by the corpora lutea after ovulation and is essential for preparing the endometrium for potential pregnancy. Elevated levels indicate successful ovulation,

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while low levels may signal reproductive issues (Lonergan & Sánchez, 2020). Progesterone concentration increased with the stage of FF collection, which is important for oocyte maturation and overall IVF (Alrabiah et al., 2023). FF concentration of hormones acting as an important character in follicle development, ovum development, and reproductive outcomes. Also, although testosterone is primarily recognized as a male hormone, it plays a role in female reproductive health, affecting indirectly ovarian function and maturation of the oocyte (Evans et al., 2022). The results show that Komar and his colleagues (2001) concluded that a decrease in estrogen concentration in bovine FF occurs because testosterone conversion to estrogen decreases, respectively, in rhythm with the decline in aromatase enzyme (Komar et al., 2001). Finally, TSH regulates thyroid hormones that are vital for maintaining metabolic processes necessary for reproductive health (Alexander et al., 2017). A study conducted to examine the role of TSH and its hormones in FF in correlation to the quantity of ova harvested in humans used in the IVF protocol procedure. This study concluded that there is a correlation between gonadal FF and thyroid hormones (Rosales et al., 2020). Understanding how these hormones interact within different-sized follicles can provide insights into fertility management in bovine species, potentially leading to improved reproductive outcomes.

Based on our knowledge, there was no study conducted to evaluate the FF hormone content in local Iraqi cattle derived from the slaughterhouse. Therefore, we initiate our hypothesis that some reproductive hormones may be altered in relation to follicle diameters. The objective of our research is to explore the hormonal profile of follicular fluid in ovarian follicles of varying sizes in Iraqi cows.

2 Material and Methods

All the ethical requirements were approved according to Al-Qasim Green University's ethical committee (#3, 3-5-2024). This study was conducted in July and August, utilizing 22 samples from sexually mature, disease-free, non-pregnant Iraqi cows sourced from a slaughter facility in Al-Hindiyah sub-governorate, Karbala, Iraq. FF was collected from ovarian follicles using sterile syringes (23-gauge), following the visual measurement of follicle diameters with an electronic digital caliper (0–150 mm accuracy). The harvested FF was subsequently transferred into sterile plastic tubes and maintained in an ice container until transport to the laboratory for hormonal analysis. The hormonal examinations were performed at Ibn al-Haytham Laboratory, employing commercially available kits: progesterone (cat# (MBS704979 detection range 1–70 ng·mL^{-1} sensitivity 0.02 ng·mL^{-1}, intra assay CV <15%, inter-assay CV <15%), estrogen (cat# MBS700251detection range 40–1,000 pg·mL^{-1} sensitivity less than 40 pg·mL^{-1}, intra assay CV <15%, inter-assay CV <15%), testosterone (cat# MBS704341 detection range 0.1–20 ng·mL^{-1} sensitivity less than 0.05 ng·mL^{-1}, intra assay CV <15%, inter-assay CV <15%), and TSH hormone kit cat# MBS2127158 detection range 24–15,000 pg·mL^{-1} sensitivity less than 11 pg·mL^{-1}, intra assay CV <10%, inter-assay CV <12%), respectively) (My BioSource, San Diego, CA, USA). Three size groups of follicles were categorized: (2–5 mm), (6–9 mm), and (10–15 mm) (Kor, 2014).

The general linear model was used to examine the effect of the hormonal profile of FF on ovarian follicles of varying sizes in Iraqi cows, using the SAS program (SAS, 2012). Regarding the significance between means, the Duncan multiple range test was used in this study with a significance level (p <0.05) (Duncan, 1955).

3 Results and Discussion

The statistical analysis of the current research found that there is a significant difference (p <0.0001) between follicle size (6–9 mm) in progesterone concentration compared to 10–15mm and 2–5 mm (35.81 ±0.42, 24.07 ±0.40, and 16.54 ±0.44 ng·ml^{-1}), respectively (Figure 1). Also, progesterone concentration was greater in medium-size 6–9 mm follicles as compared to 2–5 mm (Figure 1). Typically, the progesterone is produced from the corpus luteum in luteal phase; however, during follicle development, progesterone is produced as well. During the follicular phase, theca and granulosa cells of cow follicles synthesize huge concentrations of progesterone, which is a precursor of testosterone, and as a result, estrogen production (Barros et al., 2015). In the FF, Progesterone concentration differs through different stages of the bovine cycle, with greater concentration through the first luteal stage (Obr & Edwards, 2012). A reduced amount of progesterone concentration was noted with the rise in follicle size (Allrich, 1994). Low concentration of FF progesterone in the preovulatory follicles is due to an increase in the production of prostaglandin F2α (Berisha et al., 2024) or estrogen conversion (Hansel & Convey, 1983). Our current research is the same as what other scientists concluded in previous research, that progesterone concentration in the FF of local Iraqi cows might be useful to predict bovine fertility. Understanding these interactions can help elucidate the underlying mechanisms of fertility or infertility in cows. Monitoring hormone levels can aid in diagnosing reproductive disorders such as cystic ovaries or anovulation. For instance, low progesterone levels post-ovulation may indicate insufficient luteal function, impacting fertility outcomes.

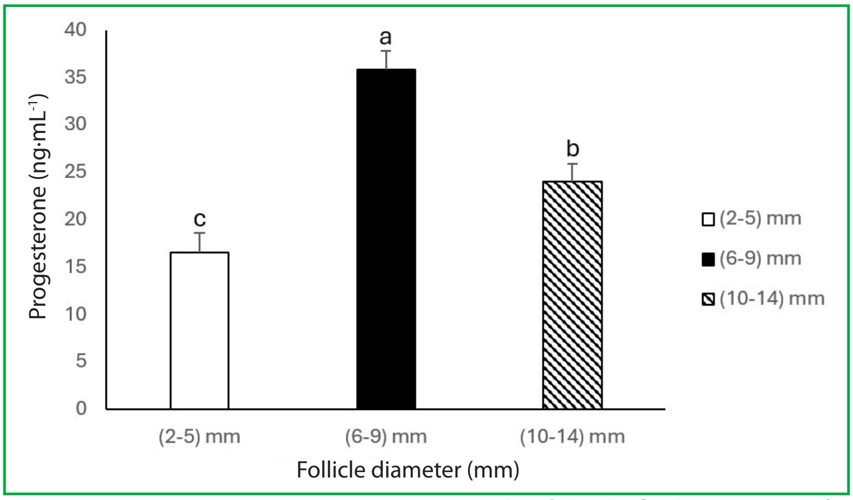

Figure 1 Progesterone concentration (ng·mL^{-1}) in follicular fluid (mean ±SEM) for three different follicular sizes (2–5, 6–9, and 10–14 mm)
Progesterone concentration increased (p <0.0001) in the 6–9 mm follicle size group compared to the 2–5 mm and 10–15 mm groups. Superscripts with different letters are significantly different

recognition (Ozturk & Demir, 2010). The current research followed what Read and his colleagues suggested, that the progesterone and estradiol concentration in the FF is correlated with preovulatory follicle size and oocyte maturation in beef cattle (Read et al., 2022). In addition, Kruip & Dielemon found that follicle selection can be determined through the concentrations of steroid hormones in the FF, which might be a possible process to predict ovum quality according to different follicle diameters (Kruip & Dieleman, 1985). Likewise, other bovine species, estrogen concentration in the FF for local Iraqi bovine might be used as a predictor of ovum quality and then successful fertility.

Estrogen concentration in the cattle FF showed a greater significance difference (p <0.0001) in follicle size 10–15 mm compared to the other two follicle sizes (6–9 mm and 2–5 mm) (0.54 ±0.02, 0.16 ±0.008, and 0.24 ±0.01 ng·ml^{-1}), respectively (Figure 2). Also, estrogen concentration was greater in 2–5 mm follicle size compared to 6–9 mm (Figure 2). The source of estrogen is the ovarian preovulatory follicle, which is controlled by the 'two-cell, two-gonadotropins" mechanism in cattle (Hillier et al., 1994). Estrogen has many roles, such as influencing estrous behavior, altering estrogen receptors (Habeeb et al., 2023), Luteinizing hormone surge, and sperm transportation (Perry et al., 2023). A larger follicle, but not a smaller one, usually produces a large amount of estrogen, because of the large number of granulosa cells in the mature follicle is necessary for LH surge (Adriaens et al., 2019), ovulation (Perry et al., 2023), and embryo attachments and

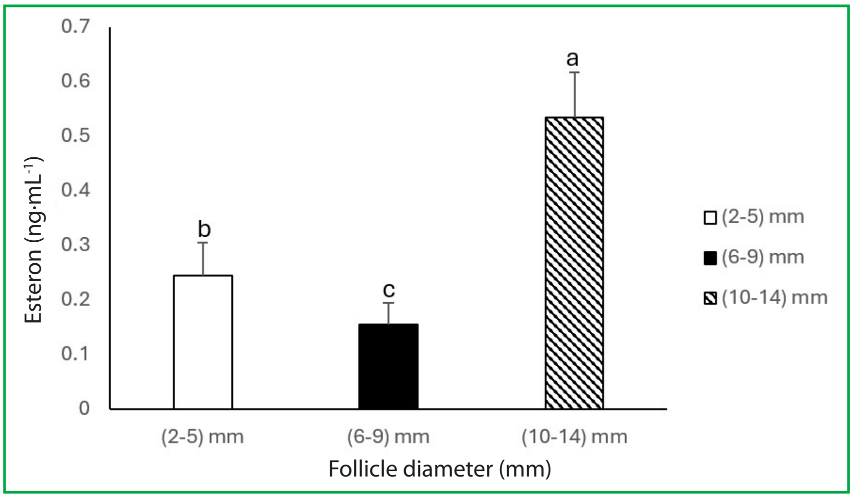

Figure 2 Estrogen concentration (ng·mL^{-1}) in follicular fluid (mean ±SEM) for three different follicular sizes (2–5, 6–9, and 10–14 mm)
Estrogen concentration was greater (p <0.0001) in the 10–15 mm follicle size group compared to the 2–5 mm and 6–9 mm groups. Superscripts with different letters are significantly different

The statistical analysis of the current research found that there is a significant difference (p <0.0001) between follicle size (2–5 mm) in testosterone concentration compared to 10–15 mm and 6–9 mm (5.28 ±0.11, 3.71 ±0.23, and 3.75 ±0.11 ng·ml^{-1}), respectively (Figure 3). However, testosterone concentration did not significantly differ between 10–15 mm and 6–9 mm follicular size in cattle (Figure 3). Testosterone is primarily recognized as a male hormone; however, it plays a role in female reproductive health, affecting indirectly ovarian function and maturation of the oocyte (Evans et al., 2022). The current result was under what Komar and his colleagues (2001) concluded that a decrease in testosterone concentration in bovine FF because of its conversion to estrogen, in rhythm with the increase in aromatase enzyme (Komar et al., 2001). Testosterone might enhance the synthesis and production of estrone and then affect follicular dynamics (Díaz et al., 2015).

Thyroid stimulating hormone concentration in the cattle FF showed greater significant differences (p <0.0001) in follicle size 2–5 mm compared to (10–15 mm) but did not

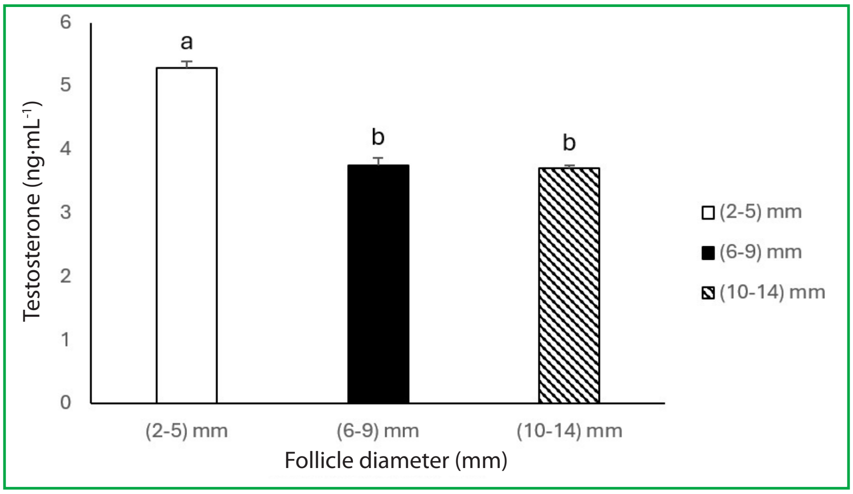

Figure 3 Testosterone concentration (ng·mL^{-1}) in follicular fluid (mean ±SEM) for three different follicular size (2–5, 6–9, and 10–15 mm)
Testosterone concentration was greater (p <0.0001) in the 2–5 mm follicle size group compared to the 6–9 mm and 10–15 mm groups. Superscripts with different letters are significantly different

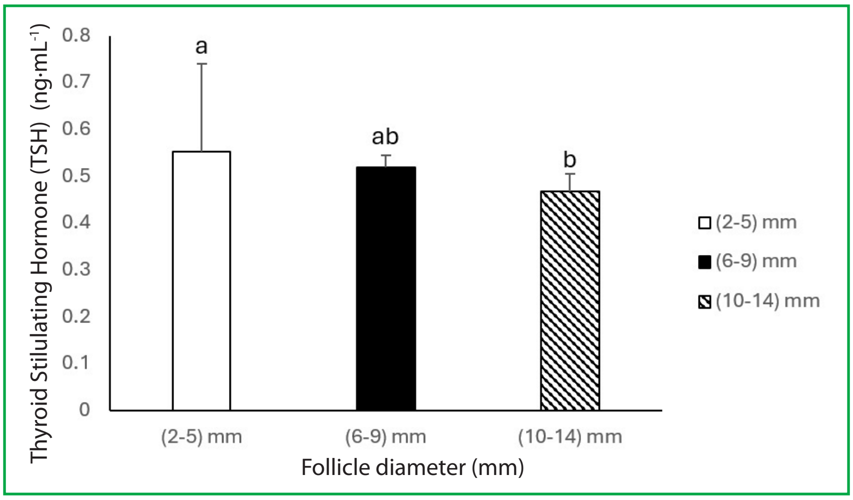

Figure 4 Thyroid-stimulating hormone concentration(ng·mL^{-1}) in follicular fluid (mean ±SEM) for three different follicular sizes (2–5, 6–9, and 10–15 mm)
Thyroid-stimulating hormone concentration was greater (p <0.0001) in the 2–5 mm follicle size group compared to the 10–15 mm group, but did not differ from the 6–9 mm group. Superscripts with different letters are significantly different

differ from 6–9 mm) (0.55 ±0.05, 0.47 ±0.00, and 0.52 ±0.00 ng·ml^{-1}), respectively (Figure 4). TSH regulates thyroid hormones that are vital for maintaining metabolic processes necessary for reproductive health (Alexander et al., 2017). The current outcome was agreed with Rosales and his coworkers, who concluded that the TSH and its hormones in FF are correlated with the quantity of ova harvested in humans used in the IVF protocol procedure (Rosales et al., 2020). In addition, in dairy cattle, TSH and T4 during early pregnancy increased, illustrating the importance of the stimulatory effect of TSH on pregnancy (Steinhoff et al., 2019). Therefore, dysregulation of thyroid hormones might affect the total reproductive outcome concerning abnormal TSH. This area of study not only enhances our understanding of bovine reproduction but also has implications for improving breeding programs through better management of hormone levels during different stages of follicle development.

4 Conclusions

In conclusion, determining ovarian FF hormone concentrations offers significant visions into reproductive physiology in bovines. Determining the variance of such hormones correlated with follicle size allows scientists to gain a deeper understanding of development approaches and fertility dynamics, ultimately improving reproductive proficiency in the cow industry. Knowing the interaction between hormonal environments and follicular diameter in females is essential for improving IVF procedures in bovines. Enhancing our understanding of these critical factors can enhance ovum pickup, fertility, and embryo quality, which can lead to effective outcomes of assisted reproductive techniques. This will progress reproductive management approaches in bovine, ultimately enhancing productivity and efficiency in the livestock industry.

Authors contribution

Hayder MH. Habeeb, Conceptualization, Formal analysis, Software, Supervision, Writing – original draft, Writing – review and editing. Amal FL Alabedi: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Software, Supervision, Visualization, Writing – original draft, Writing – review and editing.

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