Impact of Environmental Heat Changes on Corpus Luteum Parameters and Progesterone Concentration in Holstein Dairy Cows in Egypt

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The present study was conducted during two distinct seasons on 118 Holstein cows in a dairy farm located in Ismailia Governorate, Egypt. Climatic data obtained from the Meteorological Authority in Cairo were used during the period from 2023 to 2024. All cows were subjected to the Ovi-synch protocol with fixed-time insemination. Trans rectal ultrasonography was used to examine all cows. On days 10 to 13 after AI, the ovaries of all cows were carefully scanned for CL evaluation. Blood samples were collected on days 18-21 after the AI for progesterone assay. The mean CL diameter, area, and grayscale were significantly lower than their corresponding parameters recorded during winter. Furthermore, a clear negative correlation between THI and different CL parameters was observed. Summer conditions exerted a highly significant decline ($P<0.001$) in progesterone levels in cows that became pregnant compared to those in winter. In cows that failed to be pregnant, there was no significant difference ($P > 0.05$) in progesterone concentrations in cows of two groups. THI was negatively correlated with progesterone levels ($P < 0.01$), suggesting elevated THI characteristics of the summer season are associated with a decrease in serum progesterone levels. Progesterone concentration measured at the end of the cycle in cows that became pregnant showed a positive correlation with CL parameters. These correlations indicated that higher progesterone concentrations are associated with increased CL diameter, area, and volume, suggesting a potential relationship between elevated progesterone and enhanced luteal function.

Keywords: Heat stress, Cows, Corpus Luteum, Progesterone

Introduction

Heat stress (HS) through elevated average temperatures negatively influences livestock productivity and fertility ***(Cartwright et al., 2023)***. Holstein-Friesian cattle are particularly sensitive to HS, which detrimentally impacts both milk yield and reproductive efficiency ***(Lee et al., 2023)***. Heat stress arises when ambient temperatures elevate an animal's body temperature beyond its thermos neutral zone ***(Liu et al., 2020)***. The temperature-humidity index (THI), which integrates temperature and humidity, is widely used as a worldwide indicator of HS in dairy cows ***(Collier et al., 2011)***.

Heat stress impaired fertility and reproductive efficiency by lowering conception rates, increasing early embryonic loss and abortion rates ***(Nanas et al., 2020)***. The most obvious way that HS interferes with reproduction is by damaging ovarian follicles. HS not only disrupts the dynamics of follicular development throughout the oestrous cycle but also alters the growth and maturation of follicles. Consequently, corpus luteum (CL), which forms from the ovulated follicle, is similarly compromised due to the preceding follicular disruptions ***(Nardone et al., 2010)***.

Luteal insufficiency has been linked to decreased fertility in cows. It is the condition of CL that is unable to release enough progesterone to support pregnancy. During heat stress, luteal insufficiency is the result of insufficient luteinisation of the preovulatory follicle ***(Bridges et al., 2005)***.

Ultrasonography (US) may provide data regarding the physiological stage of the CL during the oestrous cycle in cows. B-mode US is used routinely to measure the luteal diameter, area, and volume. These parameters correlated with the levels of progesterone produced ***(Pirokad et al., 2022; Sharawy et al., 2023)***. Furthermore, the US can be used for measuring the CL pixel (grey scale). The pixel intensity determined in US images could be an accurate predictor of CL endocrine function and its activity throughout the CL lifespan ***(Davies et al., 2006)***. Recently, image processing and quantification have become increasingly essential in cattle reproductive management. With the widespread use of US, there is a growing need for supportive tools such as Image J to enhance image analysis, because the interpretation of US images remains time-consuming, labor-intensive, and prone to subjectivity and error ***(Gratz et al., 2020)***.

Research that investigated how subtropical heat changes influence the ovarian dynamics, CL during mid-cycle, and progesterone levels at the end of the cycle in Holstein dairy cows under Egyptian conditions is scarce. Moreover, the association between heat stress and the quality of CL and production of progesterone in dairy cows is not completely understood, that's why the objective of the present study is to clarify and to find a relationship between heat stress in different seasons and CL parameters as well as progesterone concentration in dairy cows under Egyptian condition.

Materials and methods

The present study was conducted in a dairy farm of Holstein cows located in Ismailia Governorate, Egypt. The study was carried out during two distinct seasons, winter (December, January, and February) and summer (June, July, and August) from 2023 to 2024.

1. Animals and Management

A total of 118 Holstein cows were used with an average body condition score (BCS) of 3.21 ± 0.05. The age ranged from 3 to 7 years. The cows were kept in traditional farms in free, semi-shaded yards equipped with large overhead electric fans and fed on a balanced total mixed ration (TMR). According to ***NRC (2001)***, the TMR was adjusted to fulfill or surpass the optimal requirements for milk production and BCS. Cows were milked every 8 hours, and the average 305-day milk production was about 10 tons/milking season. Regular reproductive tests and veterinary health checks were performed on the cows.

The study followed the guidelines of Ethics and Animal Experimentation Committee of Suez Canal University with registration number (2020055). This work was reviewed and approved by the Animal Care and Welfare Committee of Suez Canal University, Egypt (ANWD-206).

2. Meteorological data

Climatic data obtained from the Meteorological Authority in Cairo were used during the period from 2023 to 2024. The information included monthly averages of daily mean temperatures (AT) in Celsius degrees and mean relative humidity (RH) as a percentage for all periods of study to calculate the mean temperature-humidity index (THI) ***(Altyeb et al. 2025)***.

3. Reproductive management

All cows were subjected to the Ovi-synch protocol with fixed-time insemination after a voluntary waiting period (50 days). The cows received 10 μg Buserelin (GnRH) (Fertiboost®, 1ml contains Buserelin 4.0 μg corresponding to 4.2 μg Buserelin acetate, Marcyrl Animal Health, Egypt) intramuscularly at day 0, followed 7 days later by an intramuscular injection of 500 μg Cloprostenol (PGF2α) (Stimestrus®, 1ml contains Cloprostenol Sodium 263 μg equivalent to 250 mcg Cloprostenol base, Marcyrl Animal Health, Egypt).

After 48-56 hours from PGF2α, an additional GnRH dose was injected. The cows were bred by artificial insemination (AI) 14–16 hours after the second injection of GnRH. AI was carried out using a recto-vaginal technique with imported frozen semen of proven Holstein sires ***(Sharawy et al., 2022)***.

4. Ultrasonographical examination

Trans-rectal ultrasonography (B-mode, linear trans rectal transducer, 7.5 MHz, Sonoscape E1, China) was used to examine all 118 cows involved in the intensive study. On days 10 to 13 after AI, the ovaries of all cows were carefully scanned for CL evaluation ***(Siqueira et al., 2019)***. To obtain a better definition of the CL, once the ovary was visualized, the image was adjusted and then frozen.

In all cases, images were saved in the ultrasound machine and in an external hard disk. Stored images of the CL were processed using ImageJ software (Visual Measure 32 for Windows, version 1.7, Rise Corporation, Sendai, Japan). Corpus luteum features (CL diameter, area, volume, and grey scale) were calculated. The measurements of the cavitary CLs area were assessed by subtracting the area of the cavity from the entire CL area ***(Sharawy et al., 2023)***. The CL volume was calculated as previously described by ***Pirokad et al. (2022)*** through the following equation;

CL volume = $\frac{4}{3}\pi r^3$, where π is 3.1416 and r is the radius.

5. Pregnancy diagnosis

Pregnancy diagnosis was performed at days 28-35 after insemination using transrectal ultrasonography (linear transducer; 7.5 MHz). Confirmation of pregnancy was done on the 60^{th} day after AI. Another confirmation of pregnancy diagnosis was done on the 75^{th} day after AI ***(Sumiyoshi et al., 2022)***.

6. Blood collection

Blood samples were collected by coccygeal venipuncture on days 18-21 after the AI, and serum was separated by centrifugation at 1500 rpm for 15 min and stored at −20°C until further utilization ***(Amaral et al., 2021)***. Progesterone levels were assayed with commercially available bovine-specific ELISA kits according to manufacturers' instructions.

7. Statistical Analysis

The statistical significance of differences between CL parameters as well as serum progesterone levels in summer and winter was compared by Student's *t* test. Data are presented as mean ± SEM. Probability values less than 0.05 were considered significant. The Pearson correlation coefficient was used to analyze correlations between specific variables. Statistical analyses were performed by the GraphPad prism® version 8.4 GraphPad Software, San Diego, CA, USA.

Results

1. Meteorological conditions

The entire summer revealed high AT and THI, which was categorized as high THI, indicating that the animals were under HS during the summer. The winter season, characterized by low AT and high RH, was thus categorized as low THI (Figure 1). Ambient temperature (31.49 ± 0.19) °C and

THI (78.98 ± 0.11) were significantly higher (*P*<0.05) in the summer compared to the winter, which exhibited an AT of (14.63 ± 0.26) °C and THI (59.09 ± 0.29).

2. Ultrasonographic findings

Ultrasonographic assessments of the different parameters of CL (10-13 days after insemination) during summer and winter seasons are illustrated in Table (1) and Figure (2). The mean CL diameter (1.68 ± 0.04 cm), CL area (3.88 ± 0.28 cm^2), and CL grey scale (80.69 ± 4.19) were significantly lower than their corresponding parameters recorded during winter (1.99 ± 0.07 cm, 6.73 ± 0.76 cm^2, and 99.45 ± 4.15), respectively. Furthermore, the mean CL volume declined significantly (*P*<0.05) in summer (2.54 ± 0.19 cm^3) compared to that in the winter season (4.64 ± 0.66 cm^3).

Furthermore, a clear negative correlation between THI and different CL parameters was observed (Table 2). THI was negatively correlated with CL diameter (*r*= -0.34, *P*<0.05), CL area (*r*= -0.37, *P*<0.05), CL volume (*r*= -0.35, *P*<0.05), and CL grey scale (*r*= -0.39, *P*<0.01). These results suggested that elevated THI values may adversely affect CL development and function, and potentially compromise luteal activity.

3. Serum progesterone levels

The mean serum progesterone levels measured between days 18 and 21 after insemination during both summer and winter for pregnant and non-pregnant cows were summarized Figure (3). Summer conditions exerted a highly significant decline (*P*<0.001) in progesterone levels in cows that became pregnant (1.63±0.13 ng/ml) than that in winter (2.27±0.06 ng/ml). In cows that failed to be pregnant, there was no significant difference (*P*>0.05) in progesterone concentrations in cows of the two groups (0.79±0.02, 0.86±0.13 ng/ml in summer and winter, respectively).

Furthermore, THI was negatively correlated with progesterone levels (*r* = -0.44, *P*<0.01), suggesting that elevated THI characteristic of the summer season is associated with a decrease in serum progesterone levels.

4. The relationship between progesterone concentration and CL parameters (10-13 days after insemination)

As presented in Table (3), progesterone concentration measured at the end of the cycle in cows became pregnant showed a positive correlation with CL diameter (*r*= 0.56, *P*<0.05), CL area (*r*= 0.24, *P*<0.05), and CL volume (*r*= 0.65, *P*<0.05). These correlations indicated that higher progesterone concentrations are associated with increased CL diameter, area, and volume, suggesting a potential relationship between elevated progesterone and enhanced luteal function. However, no significant correlation was found between progesterone and CL grey scale, indicating that variations in progesterone levels do not influence the intensity of the CL's echogenic appearance on ultrasound imaging. This suggests that while progesterone is linked to physical characteristics of the CL, it does not necessarily affect its grey scale properties.

Table (1): *Ultrasonography assessment of CL parameters (10-13 days after insemination) of Holstein cows in summer and winter seasons*

Items	Summer	Winter	*P* value
Cl diameter (cm)	1.68 ± 0.04^{a}	1.99 ± 0.07^{b}	**
CL area (cm^2)	3.88 ± 0.28^{a}	6.73 ± 0.76^{b}	**
CL volume (cm^3)	2.54 ± 0.19^{a}	4.64 ± 0.66^{b}	*
CL grey scale	80.69 ± 4.19^{a}	99.45 ± 4.15^{b}	**

- Different superscripts within the same row demonstrate significant differences (*P<0.05, **P <0.01).

Table (2): *Correlation between THI and CL parameters of Holstein cows (10-13 days after insemination) in summer and winter seasons*

		Cl diameter	CL area	CL volume	CL grey scale
THI	*r*	-0.34	-0.37	-0.35	-0.39
	P value	*	*	*	**

- * Correlation is significant at the 0.05 level.
- ** Correlation is significant at the 0.01 level.

Table (3): *Correlation between the serum levels of progesterone in pregnant cows and CL parameters (10-13 days after insemination)*

		Cl diameter	CL area	CL volume	CL grey scale
Progesterone	*r*	0.56	0.24	0.65	0.14
	P value	*	*	*	NS

* Correlation is significant at the 0.05 level.
- NS: no significant correlation.

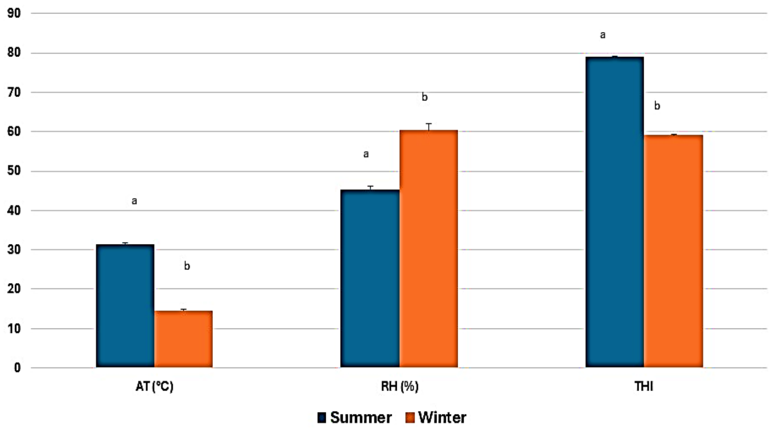

Figure (1): Mean ambient temperature (AT°C), mean relative humidity (RH%), and mean temperature humidity index (THI) during summer and winter

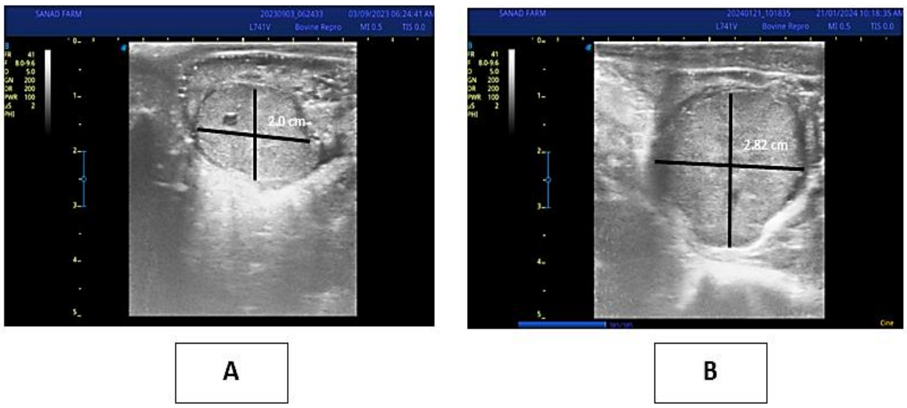

Figure (2): Ultrasonographic imaging of CL of Holstein cows (10-13 days after insemination) with scale (cm) in summer (A) and winter seasons (B)

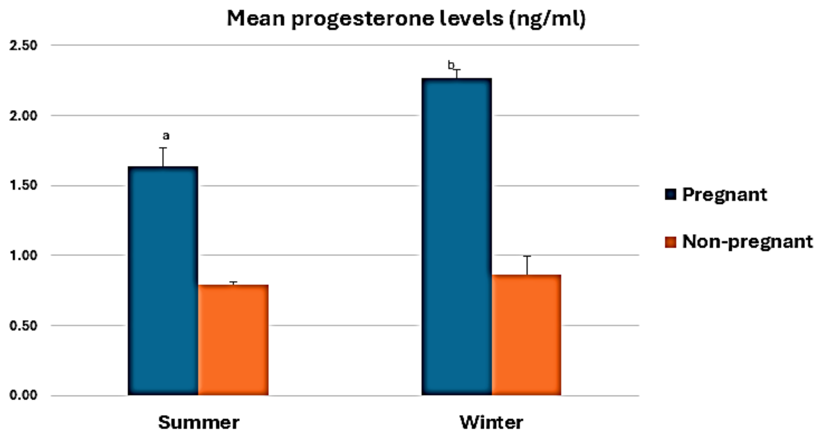

Figure (3): Serum levels of progesterone in pregnant and non-pregnant Holstein cows during the summer and winter seasons

Discussion

In Egypt, climatic conditions are stressful for the HO cows and reduce the fertility of the HO cows in summer. In the present study, THI was determined to be sufficiently high (78.98) to cause HS. during the summer season even though RH was within tolerance limits, indicating that the HS is caused due to increased AT rather than a combination of ambient temperature and RH. These findings agreed with previous findings reported by ***El-Wishy (2013)*** and ***Kumar et al. (2018)***.

Ultrasonography is important to evaluate the quality and function of CL during the mid-cycle stage and subsequently its ability to produce progesterone. Thus, measuring CL diameter, area, volume, and grey scale (pixel) may give an indication of progesterone concentration. As revealed by ***Pirokad et al. (2022)*** and Sharawy ***et al. (2023)***, CL parameters (diameter, area, volume, and pixel) positively correlated with the levels of progesterone produced, especially during the growing stage of CL. As well, plasma progesterone levels were dependent on luteal size during the mid-luteal phase (9–11 days post-ovulation) ***(Lüttgenau et al., 2011a)***.

Nevertheless, plasma progesterone concentration is determined not just by the CL's production rate, but also by the rate of secretion into the circulation. The secretion rate of progesterone is dependent on ovarian luteal blood flow, which was reported to be 30% lower during heat stress. Furthermore, possible adrenal release of progesterone, metabolism in the liver, haemodilution or haemoconcentration, the degree of heat stress, the type of heat stress (acute vs. chronic), the age of the cows, their lactation stage, and the type of feeding, all contribute to the wide variation in findings on the observed effect of heat stress on plasma progesterone concentrations ***(Trout et al., 1998)***. Therefore, CL parameters give a realistic concept of progesterone concentrations instead of plasma, serum, or milk.

In this concept, the present study showed CL parameters (CL diameter, area, volume, and grey scale) during mid-cycle were lower in values during summer in comparison with the winter season, which provides information regarding the concentration of progesterone during this stage of the cycle. These findings were similar to the results reported by ***Nanas et al. (2021a)*** regarding the CL grey scale. In seeking to explain our findings, we propose that the reduction in the size of the dominant follicle under heat stress influences the diameter of the pre-ovulatory follicle, subsequently affecting the size and development of the CL ***(Sammad et al., 2020)***. Ovulation of a smaller pre-ovulatory follicle typically results in the formation of a smaller CL. Moreover, heat stress is known to suppress LH secretion, which is essential for CL development and function. In addition, the negative energy balance (NEB) caused by decreased dry matter intake during heat stress alters metabolic hormone profiles, notably reducing insulin and IGF-I. Both hormones are vital for follicular growth and luteal cell steroidogenesis. Reduced levels of these hormones impair luteal tissue development, leading to smaller CL dimensions in terms of diameter, volume, and area during summer compared to winter (***De Rensis et al., 2017)***. Furthermore, the decline in progesterone production during heat stress may exert a negative effect on the subsequent reproductive cycle, thereby affecting the formation and functionality of the next CL. Finally, reduced ovarian blood flow in the post-ovulatory period, likely due to impaired vascularization, may further compromise CL development under heat stress conditions ***(Takahashi, 2012)***.

However, these findings are in contrast to those previously recorded by ***Jitjumnong et al. (2020)***, who recorded no differences between CL parameters (CL diameter, area, and volume) in both hot and cold seasons in beef cows. Additionally, the present study revealed that THI was negatively correlated with different CL parameters (CL diameter, area, volume, and greyscale). On the other hand, Jitjumnong ***et al. (2020)*** stated that no significant correlations were found between THI and the above-mentioned CL parameters in beef cattle.

In the current study, the estimation of the level of progesterone at the end of the oestrous cycle is used for early pregnancy detection and to evaluate the effect of heat stress on its levels during this phase. Additionally, in the present study, measuring CL parameters (diameter, area, volume, and greyscale) gave a good indication of progesterone concentrations during the mid-cycle period. The present study showed that the progesterone levels were significantly decreased in cows that became pregnant in the summer season in comparison with those in the winter season. These findings are in accordance with those reported by Amaral ***et al. (2021)***, ***Kasimanickam and Kasimanickam (2021)***, and ***Nanas et al. (2021b)***.

In this context, according to the previously mentioned findings regarding the CL parameters at the mid-cycle, all CL parameters were significantly lower in summer than in winter. Besides, the previous studies documented the positive correlation between these CL parameters and progesterone concentrations ***(Pirokad et al., 2022; Sharawy et al., 2023)***. In the same way, ***Lüttgenau et al. (2011b)*** pointed out that the evaluation of CL parameters after AI is relevant as the blood progesterone at mid-cycle depends on its size, further, ***Rizos et al. (2012)*** stated a positive association between progesterone and growing CL area.

It could be speculated that the progesterone levels might be decreased in the summer in comparison to winter in the present study. Similarly, previous studies summarized that progesterone concentrations decreased during the heat stress period ***(Ronchi et al., 2001; Alnimer et al., 2002; De Rensis et al., 2008)***.

The decline in progesterone concentration during heat stress can be ascribed to the damaged pre-ovulatory follicles, which form a CL with poor function or interruption of the CL process, or a combination of these factors ***(Chawicha and Mummed, 2022)***. Additionally, the decrease in concentrations of progesterone can be attributed to oxidative stress which disturbs follicular quality ***(Shi et al., 2016)***.

About the relationship between THI and progesterone, the present study demonstrated that THI was negatively correlated with progesterone levels. Intriguingly, the current study highlighted that the progesterone levels in cows became pregnant at the end of the oestrous cycle were positively correlated with CL diameter, area, and volume measured during the mid-cycle. ***Gómez-Seco et al. (2017)*** Claimed that the CL of pregnancy tends to grow faster than non-pregnant ones from day six to nine of the oestrous cycle.

Conclusion

It was concluded that heat stress in the summer season under Egyptian conditions may disrupt the luteal function and progesterone production in dairy cows and consequently reduce the reproductive capability in those animals. Ultrasonography, as well as image software, is a good tool to assess the quality of the corpus luteum in dairy cows.

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الملخص العربي

تأثير التغيرات الحرارية البيئية على معايير الجسم الأصفر وتركيز البروجسترون في أبقار الهولشتاين الحلوب في مصر

أجريت الدراسة الحالية خلال موسمين متميزين على 118 بقرة هولشتاين في مزرعة ألبان تقع في محافظة الإسماعيلية، مصر. تم استخدام البيانات المناخية التي تم الحصول عليها من هيئة الأرصاد الجوية في القاهرة خلال الفترة من 2023 إلى 2024. خضعت جميع الأبقار لبروتوكول Ovi-synch مع التلقيح في وقت ثابت. تم استخدام الموجات فوق الصوتية عبر المستقيم لفحص جميع الأبقار. في الأيام من 10 إلى 13 بعد التلقيح الإصطناعي، تم فحص مبايض جميع الأبقار بعناية لتقييم الجسم الأصفر. تم جمع عينات الدم في الأيام 18-21 بعد التلقيح الاصطناعي لتحليل البروجسترون. كان متوسط قطر الجسم الأصفر والمساحة والمقياس الرمادي أقل بكثير من المعايير المقابلة المسجلة خلال

فصل الشتاء. علاوة على ذلك، لوحظ وجود علاقة سلبية واضحة بين مؤشر الحرارة والرطوبة ومعايير الجسم الأصفر المختلفة. مارست ظروف الصيف انخفاضًا كبيرًا للغاية (P <0.001) في مستويات البروجسترون في الأبقار التي حملت أكثر من الشتاء. في الأبقار التي لم تحمل، لم يُلاحظ فرقٌ كبير (P>0.05) في تركيزات البروجسترون لدى أبقار المجموعتين. ارتبط مؤشر الحرارة والرطوبة سلبًا بمستويات البروجسترون (P<0.01)، مما يشير إلى أن ارتفاع مؤشر الحرارة والرطوبة في فصل الصيف يرتبط بانخفاض مستويات البروجسترون في المصل. أظهر تركيز البروجسترون المُقاس في نهاية الدورة لدى الأبقار التي حملت ارتباطًا إيجابيًا بمعايير الجسم الأصفر. أشارت هذه الارتباطات إلى أن ارتفاع تركيزات البروجسترون يرتبط بزيادة قطر ومساحة وحجم الجسم الأصفر، مما يشير إلى وجود علاقة محتملة بين ارتفاع مستوى البروجسترون وتحسين وظيفة الجسم الأصفر.