



Expression of *STAR* and *CYP19A1* Genes and Physiological Responses in Buffalo Granulosa Cells Under *in vitro* Heat Stress and Follicular Cysts Conditions



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Abstract

GLOBAL warming and heat stress are major challenges affecting livestock productivity and reproductive performance. In Egypt, buffalo milk production has declined markedly in recent years due to climate change and the limited ability of buffaloes to tolerate high environmental temperatures. Heat stress impairs ovarian function by disrupting granulosa cell steroidogenesis, leading to reduced fertility. Therefore, this study aimed to evaluate the effect of heat stress on steroidogenic activity in normal buffalo granulosa cells and to compare it with granulosa cells isolated from cystic ovaries. Granulosa cells were aspirated from normal and cystic buffalo ovaries and cultured under standard conditions until reaching 80–90% confluence. Heat stress was induced in normal granulosa cells, by incubation at 42°C for 2 h, followed by a recovery period under normal culture conditions. Total RNA was extracted, quantified using a NanoDrop spectrophotometer, reverse transcribed into cDNA, and analyzed by quantitative real-time PCR (qRT-PCR). Relative gene expression was calculated using the 2^{-ΔΔCt} method with GAPDH as the reference gene. Cell viability was assessed using a hemocytometer. Heat stress significantly reduced granulosa cell viability during both heat exposure and the recovery period. Moreover, the expression of *STAR* and *CYP19A1* was significantly downregulated, accompanied by a significant decrease in steroid hormone concentrations. In contrast, granulosa cells isolated from cystic follicles showed significantly higher expression of steroidogenic genes and increased hormone concentrations compared with normal granulosa cells. These findings demonstrate that heat stress impairs steroidogenic activity in buffalo granulosa cells, whereas cystic follicles exhibit enhanced steroidogenic activity, highlighting the distinct effects of heat stress and cystic ovarian conditions on buffalo reproductive function.

Keywords: Buffalo; granulosa cells; heat stress; cystic ovary; steroidogenesis; gene expression.

Introduction

Global warming is one of the most significant challenges affecting livestock production, particularly in subtropical regions where temperatures exceed the animals' optimal tolerance. This global warming represents a determining factor in livestock productivity due to the harsh heat elevation [1,2]. Water buffalo (*Bubalus bubalis*) is a

major source of milk production in many developing countries, including Egypt, where it plays a vital economic role in the dairy sector. However, raw buffalo milk production in Egypt has witnessed a marked decline in recent years, falling from approximately 2.9 million tons in 2014 to about 1.27 million tons in 2022 [3]. This decline is attributed to several factors, most notably rising temperatures,

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climate change, resource scarcity and inadequate strategies for adapting to heat stress [4]. Reduced farm profitability and reproductive performance are due to the negative impact on ovarian activity during the summer. Among the key cells in the ovary are granulosa cells, which play a crucial role in follicle growth and the production of steroid hormones such as estrogen and progesterone. These cells are responsible for regulating major reproductive functions such as sexual readiness and pregnancy [4]. In addition, these cells are the primary site of hormone synthesis within the follicle and provide a suitable environment for egg maturation by supplying it with nutrients, growth factors and ions [5]. Several key genes, including *STAR*, *CYP17A1* and *HSD3B2* regulate steroid activity in granulosa cells. The *STAR* protein plays a vital role in transporting cholesterol into mitochondria, a key step in initiating steroid hormone synthesis [6]. The *CYP19A1* gene crucial for steroid synthesis, provides the aromatase enzyme which is essential for estrogen production in ovarian granulosa cells. This enzyme catalyzes the conversion of androstenedione to estrone (E1) which is then converted to estradiol (E2) by 17 β -hydroxysteroid dehydrogenase enzymes, specifically *HSD17B1*. Furthermore, *CYP19A1* is involved in other processes that lead to the conversion of testosterone to estrogen. The importance of *CYP19A1* in follicular maturation, steroid synthesis and optimal ovarian function is demonstrated by the strong correlation between increased expression of this protein and elevated estradiol production during follicular growth and development in bovine ovaries [6,7]. Transzona pluripotentii (TZPs) are microscopic cytoplasmic extensions that connect granulosa cells to the oocyte via microscopic cytoplasmic extensions. They facilitate the transport of signals and nutrients and play a vital role in oocyte maturation and support. The crucial role of granulosa cells in regulating hormonal balance within the ovary reflects this synergistic gene activity. The majority of follicles undergo atrophy, and only a very small percentage (approximately 1%) reach the pre-ovulatory stage, even though a large number of follicles develop [8]. Heat stress occurs when animals are exposed to temperatures exceeding the upper critical temperature, negatively impacting physiological functions. Studies have shown that heat stress leads to complex disturbances, including increased glucocorticoid secretion, activation of programmed cell death pathways such as the FAS/FASL pathway, alterations in heat shock proteins and stress on the endoplasmic reticulum and mitochondria [9]. At the ovarian level, heat stress reduces the efficiency of oocyte maturation. In addition, it affects gene expression in oocytes and granulosa cells, which also impacts fertility. Heat stress also directly affects the

steroidogenic activity of granulosa cells leading to decreased production of hormones essential for oocyte maturation and ovulation [10]. Cystic ovarian follicles are a common reproductive problem in buffalo that directly impacting breeding efficiency. This condition disrupts the follicular development and the estrous cycle reducing fertility rates. Energy imbalances during the early lactation period, resulting from decreased feed intake and increased milk production, can also contribute to these disorders. Early diagnosis and effective management of these conditions are crucial for maintaining reproductive performance and improving economic returns [11]. This study aims to evaluate the effect of heat stress on steroidogenic activity in buffalo granulosa cells by assessing changes in their functional capacity and hormone production. It also aims to conduct a comparative analysis of the gene expression levels of *STAR* and *CYP19A1* among three groups of granulosa cells: normal cells under physiological conditions, heat-stressed normal cells and those isolated from follicular cysts.

Material and Methods

Chemicals and reagents used in this study were obtained from Thermo Fisher Scientific (California, USA) and Sigma-Aldrich (USA), unless otherwise specified.

Collection of buffalo ovaries.

This study was conducted using granulosa cells isolated from buffalo ovaries collected twice weekly from slaughterhouses during March and April ($n = 317$; on average 20 ovaries were used for each run). A total of 12 biological replicates from different collection time points were used for *in vitro* cell culture either during heat shock ($n=6$) or normal condition ($n=6$). In addition, or gene expression analysis of the four experimental groups (before HS, after HS, normal follicles, cystic follicles) was done using three biological replicates in each group.

Ovaries were selected morphologically based on the presence of the corpus luteum to confirm sexual cyclicity. Of the collected ovaries, 13 showed cystic follicles (mean follicle diameter ≥ 2.0 cm), while the remaining 304 ovaries contained normal follicles (mean follicle diameter ≤ 1.5 cm) according to our previous publication [12]. Follicle size was measured using calliper. Ovaries were moved to the laboratory in 0.9 % NaCl saline with 1.5 % antibiotics (streptomycin + penicillin) at 37 °C in a thermos within 2 hours post-slaughter. Ovaries were washed twice with pre-warmed 0.9 % NaCl, then rinsed in 70 % ethanol for 30 sec and washed again twice with pre-warmed saline solution.

Granulosa Cells Isolation and In vitro Culture

Follicular fluids were collected by aspirating antral follicles (2–8 mm in diameter) using an 18-gauge needle linked to a 10-cm syringe on average 20 ovaries/run). All cumulus-oocyte complexes were removed from the follicular fluid and discarded. The follicular fluid was transferred to a sterilized falcon tube and centrifuged at 1500 rpm for 5 minutes at 37 °C. After centrifugation, the supernatant was discarded, and the GCs clumps were dissociated using 0.25 Trypsin-EDTA in TCM-199 media supplemented with 1.5% antibiotics (streptomycin + penicillin) by gently pipetting for 5 minutes, followed by centrifugation at 1500 rpm for 5 minutes at 37°C. After removing the supernatant, the GCs pellet was resuspended in 1 ml of pre-warmed tissue culture media (TCM 199 supplemented with 10% FBS and 1.5% antibiotic). The number of GCs was counted in 100 μ l of cell suspension after incubation with 0.4% trypan blue dye for 2 minutes using a haemocytometer under an inverted microscope at 20X magnification (Leica DMI 3000B, Wentzler, Germany). The viability ratio was calculated as the percentage of living cells to the total number of cells [13].

Heat Shock of in vitro Culture Granulosa Cells

The protocol was adapted from Faheem et al., [10] with minor modifications. The number of GCs was adjusted to 250,000 cells/mL and they were grown in TCM-199 media supplemented with 10% FBS and 1.5% antibiotics (streptomycin + penicillin) in a tissue culture flask at 38.5°C and 5% CO₂ in humidified air. Once the cultivated cells became adherent and confluent (about 90%), the culture media was replenished with new medium every 2 days for up to 6 days of culture, followed by heat shock on day 7 according to experimental design. *In vitro* culture of GCs was done during the experimental period from March to May. The cultured cells were divided into two experimental groups: control and heat shock treated groups. The two control groups were *in vitro* cultured at 38.5 °C while heat shock groups were *in vitro* cultured at 42 °C for the first 2 h then transferred into control condition at 38.5 °C. The viability of cells was reassessed, and GCs were collected for further analysis using 0.25 % trypsin EDTA and stored at -80 °C until analysis of relative abundance of mRNA transcripts. In addition, the spent culture media from both heat-treated and control groups were collected from 6 different biological runs and kept at -80 °C for hormonal assays.

Hormonal Assay

Progesterone (P4) and 17 β -estradiol (E2)-levels were assessed in the spent conditioned media harvested from control and heat-treated groups by enzyme linked immunosorbent assay (ELISA) using kits specific for bovine (Sunlong Biotech CO., Ltd.; Hangzhou, China) according to the manufacturer's

procedures. Three biological samples were used to complete each analysis and the optical density (OD) was detected using a BioTek Instrument, Inc., San Diego, CA, USA, ELX808 (USA) reader at 450 nm wavelength.

Gene Expression Analysis

RNA purification and cDNA synthesis from three different biological replicates were used for RNA extraction from granulosa cells using Thermo Scientific GeneJET RNA extraction kit (Thermo Scientific, USA) according to manufacturer's instructions. The pellet of samples after centrifuging was resuspended in 600 μ l of lysis buffer and mixed by vortex. Ethanol (96-100%) was added and mixed by pipetting. Samples were transferred to the purification column inserted into the collection tube. The step of lysate was repeated until all of the lysate was transferred. Wash buffer I and wash buffer II were added. Finally, nuclease-free water was added at the centre of the column to elute the sample. The exact concentration and purity of the extracted RNA were measured by NanoDrop 2000C (ThermoFisher Scientific, USA). The extracted RNA samples that recorded a ratio ≥ 1.8 at 260/280 were used for cDNA synthesis. The reverse transcription of RNA was done using RevertAid cDNA synthesis kit (ThermoFisher Scientific, Vilnius, Lithuania). Briefly, reaction was performed by adding 4 μ l of PCR reaction buffer, 2 μ l of dNTPs, 1 μ l of oligo dT18 primer, 1 μ l of RNA inhibitor, and 1 μ l of reverse transcriptase enzyme to 11 μ l of RNA sample and mixed well by pipetting. The reaction mix was incubated at 42 °C for 60 minutes followed by 5 minutes at 70°C. The synthesized cDNA was stored at -20°C until quantitative real time PCR runs.

Quantitative Real-Time PCR

The samples were analyzed in three replicates. The relative expression of mRNA of the two biomarkers (*StAR* and *CYP19A1*) Relative mRNA expression levels were quantified by real-time PCR using Thermo Fisher Scientific Maxima SYBR Green qPCR Master Mix (2X) with ROX reference dye, according to the manufacturer's instructions (Thermo Fisher Scientific, USA). The expression levels of the target mRNA were assessed by real-time PCR and normalized to the housekeeping gene *GAPDH*. Melting curve analysis was performed to confirm the specificity of the amplified products. The primer sequences of all genes selected for this study were designed from buffalo (*Bubalus bubalis*) (www.ncbi.nlm.nih.gov) in the GenBank using database Primer3web (<http://primer3.wi.mit.edu/>). The primer sequence used for *StAR* forward 5'-CCATGGAGAGGCTTTATGAA-3' and reverse 5'-TCTTTCCCAATCTTCTGCAG-3', for *CYP19A1* forward 5'-GGCTATGTGGACGTGTTGACC-3' and reverse 5'-TGAGAAGGAGAGCTTGCCATG-3' and for *GAPDH* forward 5'-

TGCCCAGAATATCATCCCT-3' and reverse 5' CTCATCATACTTGGCAGGTT-3'. The reaction was carried out in a 20 μ l total volume with 10 μ l of Maxima SYBR Green. PCR Master Mix, 2.0 μ l cDNA, 7.8 μ l nuclease-free water and 0.2 μ l primers. The following thermal cycling conditions were used for PCR reactions: 2 minutes at 50°C, 10 minutes at 95°C then forty cycles of 15 seconds at 95°C, 20 seconds at 60°C and 30 seconds at 72°C followed by a final 1 minute at 60°C. A Step One plus TM Real-Time PCR apparatus was used to perform the real-time PCR (Applied Biosystems, USA). According to MIQE requirements, the results were quantitatively evaluated using the delta-delta CT method.

Statistical Analysis

GCs viability and metabolic activity levels were evaluated using a one-way analysis of variance (ANOVA). The mean $\pm$ standard error of means (SEM) was used to display the data. Significant differences between comparisons were indicated by P-values less than 0.05. The IBM SPSS Statistics V.27 software [14] was used for all analyses. Using the general linear model (GLM) technique, SAS 9.1 [15] was used to assess the expression profiles of chosen target genes. To find differences between means, Duncan's multiple range test was also used [16].

Results

Cell Viability

Granulosa cell viability was assessed before heat stress (Before HS), immediately after heat stress (After HS) and after one week of recovery (After HSR). Exposure of buffalo granulosa cells to heat stress (42°C for 2 h) significantly reduced cell viability compared with the control group (Before HS). Cell viability remained significantly lower after one week of recovery, indicating that heat stress exerted a sustained negative effect on granulosa cell survival (Fig. 1).

Relative Expression of Steroidogenic Genes

The relative mRNA expression of *CYP19A1* and *STAR* was evaluated in buffalo granulosa cells under different experimental conditions. Heat stress significantly downregulated the expression of both genes compared with the control group. In contrast, granulosa cells isolated from cystic follicles exhibited significantly higher expression levels of *CYP19A1* and *STAR* than granulosa cells obtained from normal follicles (Fig. 2A, B).

Steroid Hormone Concentrations

The concentrations of 17 β -estradiol and progesterone were measured in the spent culture medium of granulosa cells before and after heat stress and in follicular fluid collected from normal and cystic follicles. Heat stress significantly decreased estradiol (166.1 ± 7.0 vs. 90.5 ± 7.0

ng/mL) and progesterone (49.4 ± 3.3 vs. 23.9 ± 3.3 ng/mL) concentrations compared with the control group. In contrast, granulosa cells from cystic follicles showed significantly higher progesterone concentrations (129.8 ± 3.3 ng/mL) than normal follicles (51.2 ± 3.3 ng/mL), while estradiol concentration was also higher in cystic follicles (176.3 ± 7.0 ng/mL) than in normal follicles (162.7 ± 7.0 ng/mL) (Table 2).

Discussion

The seasonal reproductive behaviour is considered one of the most common problems affecting buffalo reproduction. The sexual activity of buffalo is stimulated by the decrease in day length and temperature. The highest rate of buffalo reproduction occurs in winter and the lowest in summer [9]. The postpartum estrous cycle in buffalo was highest in September, followed by October, while the lowest rates were in April and May which may be due to high temperatures [17]. Heat stress is defined as the major factor affect in ovarian function and fertility in livestock species. Granulosa cells are particularly essential for follicle growth, egg maintenance, and steroid hormone production; therefore, they are highly sensitive to high temperatures [18]. Consequently, follicular efficiency and reproductive capacity may be directly affected by changes occurring in granulosa cell viability. In this study viability test revealed a reduction in the count of cells after exposure to heat stress at (42°C for 2 h) suggesting the increase in heat stress may affect the cell survival mechanism increasing ROS production, activating apoptotic pathways and inducing mitochondria dysfunction in ovarian cells [6,19]. It was demonstrated that the viability of buffalo granulosa cells significantly decreased after exposure to the temperature of 42°C/2h compared with that of control cells incubated at 39°C and 40°C [10]. These findings are consistent with the data of the present study and may indicate that the granulosa cells are highly sensitive to high temperature that may affect follicular growth due to decrease cell viability. During the hot season, a marked decline in estrus expression, accompanied by lowered plasma estradiol levels in heat-stressed cows, was attributed to the impairment of the aromatase enzyme, which regulates the steroidogenic activity of GCs [20–22].

Noteworthy, the reduction in viability of granulosa cells is likely associated with alterations in the gene expression that regulate steroidogenesis. In this study, two genes (*CYP19A1* and *STAR*) related to cholesterol transport and steroidogenic enzyme activity and are necessary for steroid hormone production, were measured., *STAR* gene is essential for transporting cholesterol to mitochondria, while *CYP19A* encodes aromatase enzyme to convert androgenic substance into estrogen. The present results showed a decrease in the expression of

CYP19A1 and *STAR* genes after heat shock treatment (42°C for two hours) on cultured granulosa cells.

In consistence with [23,24], exposure of bovine GCs to heat stress led to downregulation of several genes, indicating an inhibition of the key pathways for hormone synthesis and follicular growth, this result matched with the current finding. Another study showed that severe heat stress affects granulosa cells by increasing their destruction and reducing aromatase expression in ovarian follicles. Inhibition of these genes may be a key biochemical mechanism by which heat stress impairs ovarian function. As *CYP19A1* is responsible for regulating estrogen biosynthesis, while *STAR* controls the availability of cholesterol necessary for steroid production [25]. Together with results of our investigation, it could be indicated that heat shock may negatively affect steroidogenesis pathways in buffaloes. Thermal shock caused a markedly reduction in the viability of buffalo GCs by upregulating the apoptotic—gene (*P53*) and downregulating the steroidogenic biosynthesis gene (*STAR*), which consequently elevated the expression of cellular heat shock transcription factor gene—(*HSF1*) as a compensatory mechanism to mitigate the negative effects of thermal stress [26]. Indeed, alteration of GCs steroidogenesis drastically decreased cow's sexual behavior and ovulation [27], in addition to causing anestrus and ovarian cysts in dairy cattle [28].

Taken into consideration, regulation of these genes responsible for steroid secretion is directly related to blood hormonal profile. Consequently, changes in the expression of *CYP19A1* and *STAR* transcripts altered the production and release of steroid hormones especially under severe thermal stress. The results of two different publications stated that down-regulation of estradiol and progesterone during recovery period [20–22]. Heat shock (HS) reduces the circulating estradiol concentrations in cattle by suppressing aromatase activity and inhibiting granulosa cell steroidogenesis [20,21]. Noteworthy, estradiol is essential for follicular growth, estrous activity, and ovulation. Correspondingly, diminished estradiol biosynthesis resulting from thermal stress in cows has been directly linked to lowered estrous manifestations and impaired reproductive efficiency [27]. Furthermore, heat stress has been linked to a widespread prevalence of ovarian disorders in cows, including anestrus and ovarian cyst formation [28,29], as well as silent estrus in buffalo [30] and compromise ultimate fertility rate of this species [31,18].

Consequently, one of the main impacts of heat stress on reproductive efficiency may be declined steroid synthesis. On the other hand, granulosa cells extracted from cystic follicles showed higher expression of *CYP19A1* and *STAR* genes compared to the normal counterparts. Failure of ovulation and prolonged follicular persistence, leading to hormonal

imbalance and continued metabolic activity within the follicle, are characteristic of follicular cysts. Elevated expressions of *CYP19A1* and *STAR* gene may affect aromatase activity and enhanced cholesterol transport to the mitochondria. This suggestion is supported by previous research showed a marked increase in the expression of genes important for steroidogenesis in ovarian follicular cysts, such as *STAR*, *CYP11A1*, *CYP17A1*, and *3 β -HSD* [32]. These molecular changes were closely correlated with changes in steroid hormone concentration, suggesting that the activation of steroidogenesis pathways facilitates hormonal activity and follicular permanence [32]. This research supports the link between increased steroid production and cyst formation, through the correlation between hormone levels and gene expression patterns. The limitation of the present study could be summarized in the collection season of the ovarian samples that were collected during the winter season, although the main problem occurs in summer. In addition, the study investigated the effect of the heat stress on gene expression and hormones, but did not investigate the potential strategies to reduce or solve this problem. Furthermore, only one heat stress condition (42°C) was evaluated, whereas animals in the field are exposed to different temperatures and durations of thermal stress. Accordingly, future studies are recommended on samples collected during the summer season to validate the present findings under wider variable temperature conditions to determine the severity and degree of the effect on steroidogenesis. The study of the molecular mechanisms and hormonal expression of heat stress on normal granulosa cells and cystic ovary that help to control the activity of steroidogenesis and help to find new strategies to overcome the problem of cyst and increase thermal tolerance efficiency in buffalo.

Conclusion

This study highlights the effect of heat stress and abnormal follicle size such as cystic ovary on steroidogenesis of buffalo granulosa cells. The outcomes indicate that heat stress negatively affects both the molecular and hormonal functions of GCs. In contrast, the upregulation of expression of *STAR* and *CYP19A1* in cystic ovaries that leads to increased production of estradiol and progesterone hormones. This indicates that cystic follicles maintain active steroid production pathways despite their abnormal physiological state. These findings provide a deeper understanding of the mechanisms by which heat stress and ovarian cysts affect granulosa cells function and reproductive performance in buffalo. Future studies investigating additional genes associated with steroidogenesis and thermal stress, as well as potential strategies for mitigating the effects of heat stress, are needed to further enhance our understanding of ovarian function and reproductive efficiency in buffalo.

Funding statement

This study didn't receive any funding support.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethics statement

All procedures of the present study have been approved by the Institutional Animal Care and Use Committee (IACUC) at Faculty of Agriculture, Suez Canal University, Egypt (Approval No.: SCU-Agr-REC 31/2026).

Author Contributions

All authors contributed equally to this work.

TABLE 1. Level of steroid hormones (Mean±SEM) in spent media of buffalo granulosa cells *in vitro* cultured under heat shock (42°C/2h) compared to normal control conditions (38.5 °C) and in freshly recovered follicular fluid from normal (≤ 1.5 cm) or cystic (≥2.0 cm) follicles.

Biochemical	Spent Medium		Follicular Fluid	
	<i>In vitro</i> cultured GCs before heat shock	<i>In vitro</i> cultured GCs after heat shock	Normal Follicles (≤ 1.5 cm)	Cystic Follicles (≥ 2.0 cm)
17β-Estradiol (ng/mL)	166.1±7.0 ^a	90.5±7.0 ^b	162.7±7.0 ^a	176.3±7.0 ^a
Progesterone (ng/mL)	49.4±3.3 ^b	23.9±3.3 ^c	51.2±3.3 ^b	129.8±3.3 ^a

* Values in the same row with different superscripts differ significantly ($P<0.05$); ** GCs: Granulosa Cells.

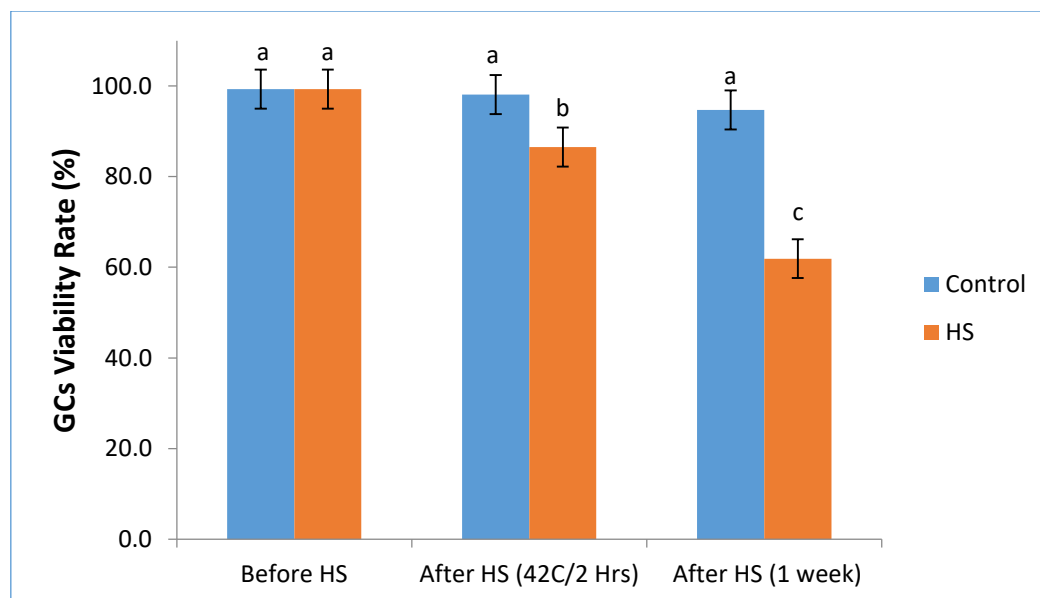


Fig.1. Viability rate of buffalo granulosa cells *in vitro* cultured under heat shock (42°C/ 2 h) and after one week recovery period (38.5 °C) from heat stress compared with normal control conditions. Bars with different superscript letters differ significantly ($P<0.05$).

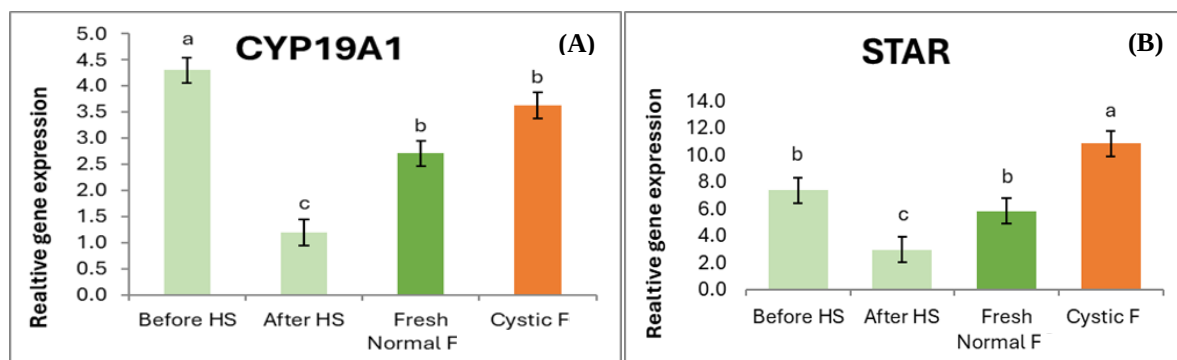


Fig.2. Relative expression *CYP19A1* (A) and *STAR* (B) genes in buffalo granulosa cells *in vitro* cultured under heat shock (42 °C/ 2 h) and after one week recovery period compared to normal control and cystic follicles conditions (38.5 °C). Bars with different superscript letters differ significantly ($P<0.05$).

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التعبير الجيني (*STAR* و *CYP19A1*) والنشاط الفسيولوجي في الخلايا الحبيبية للجاموس تحت ظروف الإجهاد الحراري المعملية والحوصلات المبيضية المتكيسة

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الملخص

يُعد الاحتباس الحراري والإجهاد الحراري من أهم التحديات التي تؤثر في إنتاجية الماشية وكفاءتها التناسلية، ففي مصر انخفض إنتاج حليب الجاموس من حوالي 2.9 مليون طن عام 2014 إلى 1.27 مليون طن عام 2022 نتيجة للتغيرات المناخية وضعف القدرة على التكيف مع الإجهاد الحراري. ويؤثر الإجهاد الحراري سلبيًا على وظائف المبيض من خلال تغيير النشاط الستيرويدي للخلايا الحبيبية وانخفاض الخصوبة. لذلك هدفت هذه الدراسة إلى تقييم تأثير الإجهاد الحراري على النشاط الستيرويدي في الخلايا الحبيبية الطبيعية للجاموس، بالإضافة إلى تقييم تأثير تكيس المبايض على هذا النشاط. تم جمع الخلايا الحبيبية من مبايض الجاموس الطبيعية ومن المبايض المصابة بالتكيس، ثم زُرعت تحت ظروف طبيعية حتى وصلت إلى كثافة خلوية تتراوح بين 80–90%. بعد ذلك، تم تعريض الخلايا المأخوذة من المبايض الطبيعية للإجهاد الحراري عند درجة حرارة 42°م لمدة ساعتين، تلاها فترة استشفاء وإعادة زراعة. تم استخلاص الحمض النووي الريبوزي الكلي (RNA) من جميع المجموعات وقياس تركيزه باستخدام جهاز النانودروب (NanoDrop)، كما تم تحويل الحمض النووي الريبوزي إلى الحمض النووي التكميلي (cDNA)، ثم تحليل التعبير الجيني باستخدام تقنية تفاعل البوليميراز المتسلسل الكمي (Real-Time PCR)، وتم تقدير مستويات التعبير الجيني النسبي باستخدام طريقة $2^{-\Delta\Delta Ct}$ مع استخدام جين GAPDH كجين مرجعي. كذلك، تم تقييم حيوية الخلايا باستخدام جهاز عد الخلايا (الهيوموسيتومتر). أظهرت النتائج انخفاضًا معنويًا في حيوية الخلايا الحبيبية بعد التعرض للإجهاد الحراري وخلال فترة الاستشفاء، كما أدى الإجهاد الحراري إلى انخفاض معنوي في التعبير الجيني لكل من *STAR* و *CYP19A1* مصحوبًا بانخفاض في تركيز الهرمونات الستيرويدية. وعلى النقيض من ذلك، أظهرت الخلايا الحبيبية المأخوذة من الجريبات الكيسية ارتفاعًا معنويًا في مستويات التعبير الجيني وتركيزات الهرمونات مقارنةً بالخلايا الطبيعية. وتشير هذه النتائج إلى أن الإجهاد الحراري يؤثر سلبيًا على الجهاز التناسلي في الجاموس من خلال تقليل النشاط الستيرويدي، في حين يرتبط تكيس المبايض بزيادة النشاط الستيرويدي.

الكلمات الدالة: الجاموس، الخلايا الحبيبية، المبيض الطبيعي، المبيض المتكيس، تكوين الهرمونات الستيرويدية، الإجهاد الحراري.