Reproductive hormones profile of Iraqi Awassi ewes immunized against synthetic inhibin-α subunit or steroid-free bovine follicular fluid

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Abstract

The current study was conducted to investigate the impacts of active and passive immunization against synthetic inhibin-α subunit and steroid-free bovine follicular fluid, respectively, on reproductive hormones profile out of breeding season in Iraqi Awassi ewes. Follicular fluid was aspired from mature bovine follicles, treated with activated charcoal, used for immunization of male rabbits, and obtaining of SFBFF antiserum. Forty non-pregnant Awassi ewes were allocated into 4 groups (n = 10 each). At day 38 of experiment, ewes were treated with intra-vaginal sponge impregated with medroxyprogesterone acetate 60 mg for 12 days. Ewes were treated at 0, 28 and 50 days with 4, 2 and 2 ml of normal saline (control; C-ve), 400, 200 and 200 µg of ovalbumine (C+ve), 400, 200 and 200 µg of inhibin (SI group), and 4 ml of normal saline at 0 day, and 4ml and 2ml of SFBFF antiserum, at 28 and 50 days, respectively (AI group). Blood samples were collected at 24 and 48 hours before and after sponge withdrawal for assessment of FSH, LH, inhibin-B, Activin-A, E2 and P4. Before sponge withdrawal, FSH level increased in SI ewes, whereas only after sponge withdrawal, FSH, LH, activin-A and E2 levels increased in SI and AI ewes. Opposite results were shown of inhibin-B level. In conclusion, active or passive immunization against inhibin in Awassi ewes could augment reproductive functions out of breeding season in Iraqi Awassi ewes.

Keywords: Active Immunization, Passive Immunization, Follicular Fluid, Superovulation, Ewes, Reproduction
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صورة الهرمونات التكاثرية للنعاج العواسية الممنعة ضد وحدة الانهبين ألفا المصنع و السائل الجريبي البقري منزوع الستيرويدات

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الخلاصة

أجريت الدراسة الحالية للتحري عن تأثير التمنيع الميسر والفعال ضد وحدة الانهبين ألفا المصنع والسائل الجريبي البقري منزوع الستيرويدات، على التوالي، على صورة الهرمونات التكاثرية خارج موسم التناسل في النعاج العواسية. تم شفط السائل الجريبي من الجريبات البقرية الناضجة وعوملت مع الفحم النشط واستخدمت لتمنيع ذكور الأرانب ومن ثم الحصول على مضاد والسائل الجريبي البقري منزوع الستيرويدات. وزعت النعاج العواسية على أربع مجموعات (١٠ لكل مجموعة). في اليوم ٣٨ من التجربة يمت معالجة النعاج بالاسفنجات المهبلية المشربة مع الميدروكسي برجستيرون أسيتات ٦٠ ملغم لمدة ١٢ يوم. عوملت النعاج في الأيام ٠ و ٢٨ و ٥٠ بـ ٤ و ٢ و ٢ مللتر من السائل الفسلجي (السيطرة السالبة) و ٤٠٠ و ٢٠٠ و ٢٠٠ مايكرغرام من ovalbumine (السيطرة الموجبة) و ٤٠٠ و ٢٠٠ و ٢٠٠ مايكروغرام من الانهبين (مجموعة SI) و ٤ مللتر من السائل الفسلجي في اليوم صفر و ٤ و ٢ مللتر من مضاد SFBFF في اليومين ٢٨ و ٥٠ ، على التوالي (مجموعة AI). تم جمع عينات الدم ٢٤ و ٤٨ ساعة قبل وبعد رفع الاسفنجات المهبلية لغرض قياس تركيز الهرمون محفز الجريب و الهرمون المصفر و الانهبين- بي والاكتفين-أي والاسترادايول والبروجستيرون. قبل رفع

الاسفنجات المهبلية ازداد مستوى FSH في مجموعة SI بينما بعد رفع الاسفنجات المهبلية ازداد مستوى كل من FSH و LH و Activin-A و الاسترادايول في مجموعتي SI و AI. عكس النتائج ظهرت بالنسبة لـ Inhibin-B. يمكن الاستنتاج أن التمنيع بنوعيه الميسر والفعال ضد الانهبين في النعاج العواسية يعزز من الوظائف التكاثرية خارج موسم التناسل.

Introduction

Awassi breed is a monotoccus with low ovulation rate and low incidence of twinning (1,2). To increase fecundity in sheep, many non-genetic methods have been used, including administration of exogenous gonadotropins such as equine chorionic gonadotropin (3), passive and active immunization against ovarian steroids (4) and, recently, passive immunization against endogenous inhibin (5).

Superovulation has been encouraged successfully by passive immunization against alpha subunit of inhibin (6-9), and active immunization against synthetic inhibin (10). Improved reproductive action of mammals requires promotion of pituitary FSH secretion to authorize ovarian small follicles for development. In this circumstance, it is required to have surge of FSH secretion to augment follicular development to the point where LH receptors formed inside granulosa cells of the growing follicle. The key point for FSH surge is high levels of hypothalamic GnRH and pituitary activin, whereas the negative regulator is gonadal inhibins (11).

There are many attempts have been used to use for successfully to induce multiple ovulations, such as pig pituitary purified FSH and equine placenta purified equine chorionic gonadotropin; eCG. These products have been used as practical application in an embryo transfer program. The short half-life of FSH (12,13) and long half-life of eCG and induction of endogenous anti-eCG antibodies (14) were the main side effects of these programs. So the regulatory effect of inhibin by way of passive or active immunization has been tested as an alternative method to increase FSH level and to induce ovarian follicular development in numerous species, including sheep (15,16), cows (17), goats (18), mice (7), and rats (5,19).

The objective of current investigation was to evaluate the role of active and passive immunization against inhibin, as alternative methods, to improve ovulation rate and reproductive hormones profile during estrus cycle and gestation period in Iraqi Awassi ewes.

Material and methods

Preparation of synthesis of inhibin

Synthetic peptides [NH_2] with a Sequence chgleldrelvlakvralfldalghppvt [COOH], was obtained from Bio-synthesis Inc., USA, as 5mg of purified synthetic inhibin powder.

Preparation of Steroid-free bovine follicular fluid (SFBFF)

Bovine follicular fluid was aspired from bovine ovarian follicles (≤15mm in diameter) and centrifuged at 8000 rpm for 15 minute at 4°C to remove cellular debris. Activated charcoal (10 mg/ ml) was added to the FF, mixed for 1 hour at 4°C and centrifuged at 14000 rpm for 90 minute at 4°C to remove charcoal with steroid to obtain steroid-free BFF. SFBFF was kept at -20°C until use (19).

Preparation of SFBFF antiserum

Ten mature male rabbits have been injected for 5 times with 1 ml of SFBFF subcutaneously (sc.) one week interval. One month after the last injection, blood was collected, centrifuged and antiserum was obtained and kept at –20 C° until use(19).

Animals

Awassi ewes aged 2.5-3.5 years and weighed 50-58 kg were used in the present experiment. The animals were housed at night, while, freely feeding grass throughout the remaining hours of the day. Indoors, the ewes were fed concentrated feed supplemented with vitamins and wheat straw, water and minerals were freely available.

Experimental design

Forty non-pregnant mature Awassi ewes were allocated into 4 groups (10 each). At treated at 38th day of experiment, Awassi ewes were treated with intra-vaginal sponge impregnated with medroxyprogesterone acetate (MPA) for 12 days. First group ewes were treated at 0, 28th, and 50th days of experimental periods with subcutaneous injections of 4, 2 and 2 ml of normal saline and served as negative control group (C-ve). Second group ewes were treated at 0, 28th, and 50th day of experiment with sc injections of 400, 200 and 200 µl of ovalbumine and served as positive control group (C+ve). Third group ewes were treated at 0, 28th, and 50th day of experiment with sc injections of 400, 200 and 200 µl of synthetic inhibin and served as SI treated group (SI). Forth group ewes were treated at 0 day with sc injection of 4 ml of normal saline, and 4ml and 2ml of SFBFF antiserum, at 28th, and 50th day of experiment and served as SFBFF antiserum treated group (AI). Blood samples were collected in non-heparinized tubes every 24 hours, started at 48 hours before and lasted at 48 hours after sponge withdrawal. Blood sera were separated and kept at -22 ºC until hormonal assessment of FSH, LH, inhibin-B, Activin-A, E2 and progesterone concentrations, using ELISA technique

depended on the manufacturer instructions (Wuhan Fine Biological Technology).

Statistical Analysis

Results were expressed as mean ± standard deviation. Comparisons between groups and periods values were performed using two way analysis of variance (ANOVAII) and Newman- Keuls. Differences were considered to be significant at the level of $P<0.05$ (20). Statistical analysis was carried out using the GraphPad Prism version-5 (GraphPad Software, Inc. California, USA).

Results

The results of the current study confirmed the potency of passive and active immunoneutralization against endogenous inhibin, in Awassi ewes out of season breeding, to enhance the surge of FSH secretion from pituitary gland and to elevate gonadal estradiol biosynthesis by follicular cells.

FSH

In active immunized (SI) group ewes, serum FSH concentration registered significant increase ($p<0.05$) before 48hrs and 24hrs of sponges withdrawal compared with other groups ewes, which showed no significance difference ($p>0.05$) between each other during these periods. After sponge withdrawal (after 24hr and 48hr), the concentrations in SI and AI group ewes elevated significantly ($p<0.05$) than control (C-ve and C+ve) group ewes. In comparison between periods for each group, FSH concentrations of all group ewes elevated significantly ($p<0.05$) after sponge withdrawal, but the elevation in SI and AI group ewes reached 2 times more than that of control groups (figure 1).

LH

Serum LH concentrations revealed no significant ($p>0.05$) differences among experimental group ewes during the periods before sponge withdrawal (before 48hr and 24hr periods). After sponge withdrawal, the concentrations in SI and AI group showed significant ($p<0.05$) elevation than both of control group ewes. In comparison between periods, all group ewes showed gradual significant ($p<0.05$) elevation of serum LH concentrations along with the progress of experiment periods, but the elevation in immunized groups (SI and AI) was significantly ($p<0.5$) higher than control groups (figure 2).

Inhibin-B

As illustrated in figure (3), inhibin-B concentration showed significant decrease ($p<0.05$) in SI group ewes, at both periods before sponge withdrawal, in comparison with other groups. At periods of sponge withdrawal, the concentrations of inhibin-B still significantly down-regulated in SI group ewes, where that of AI group ewes also declined significantly ($p<0.05$) than control groups as well as SI group after 24hr period of sponge withdrawal, but it showed no significant ($p>0.05$) difference compared with SI group ewes after 48hr of sponge withdrawal. In comparison between periods, in SI group ewes, the concentrations remained insignificant ($p>0.05$) at all experimental periods, whereas that of other groups declined gradually along with experimental progress.

Activin-A

Serum concentrations of activin-A in SI and AI group ewes reported significant ($p<0.05$) higher levels than other experimental groups during all periods of the experiment, where they showed gradual elevation along with the progress of the experiment until 24hr after sponge withdrawal, then decreased after 48hr of sponge withdrawal. In AI group ewes, serum activin-A concentration was significantly ($p<0.05$) higher, at 24hr and 48hr after sponge withdrawal, than that of SI group ewes at the same periods (figure 4).

Estradiol-17B (E2)

The results of serum estradiol-17B concentrations clarified in figure (5), showed significant ($p<0.05$) elevation in SI group ewes followed by AI group ewes at 48hr and 24hr periods before sponge withdrawal, whereas that of control groups reported insignificant ($p>0.05$) difference between each other. After 24hr and 48hr of sponge withdrawal, the concentrations of E2 in all groups ewes were significantly ($p<0.05$) elevated, but still the concentrations of SI and AI group ewes were significantly ($p<0.05$) higher than that of control.

Progesterone

The results demonstrated in figure (6) revealed no significant ($p>0.05$) difference between experimental groups at 48hr and 24hr before sponge withdrawal, but significant decline was shown in SI and AI group ewes at 24hr and 48hr after sponge withdrawal compared with control groups. In comparison between periods, all groups reported gradual decline with the progress of the experimental periods.

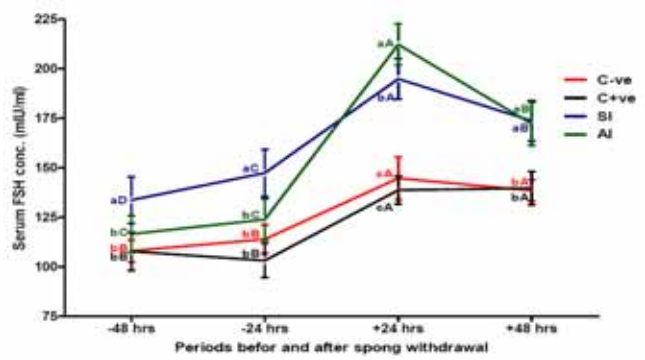

Figure 1: Serum FSH concentration (mIU/ml) before 48 and 24 hrs. (A and B) and after 24 and 48 hr. (C and D) sponge withdrawal at day 50 of treatment in Awassi ewes injected, at 0, 28, and 50 days of treatment, with 4, 2 and 2 ml of normal saline (control; C-ve), 400, 200 and 200 µg of ovalbumine (positive control; C+ve), 400, 200 and 200 µg of synthetic inhibin (SI group), and 4 ml of normal saline followed by 4 and 2 ml of SFBFF antiserum (AI group). The values represented as M±SD. Different small letters denote significant difference ($p<0.05$) between groups for each period. Different capital letters denote significant difference ($p<0.05$) between periods for each group.

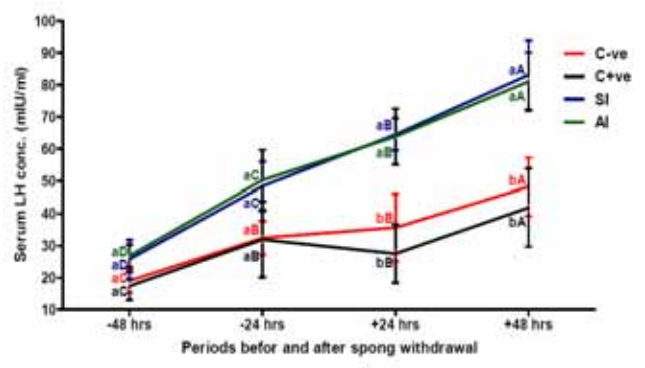

Figure 2: Serum LH concentration (mIU/ml) before 48 and 24 hrs. (A and B) and after 24 and 48 hr. (C and D) sponge withdrawal (at day 50 of treatment) in Awassi ewes injected, at 0, 28, and 50 days of treatment, with 4, 2 and 2 ml of normal saline (control; C-ve), 400, 200 and 200 µg of ovalbumine (positive control; C+ve), 400, 200 and 200 µg of synthetic inhibin (SI group), and 4 ml of normal saline followed by 4 and 2 ml of SFBFF antiserum (AI group). The values represented as M±SD. Different small letters denote significant difference ($p<0.05$) between groups per each period. Different capital letters denote significant difference ($p<0.05$) between periods per each group.

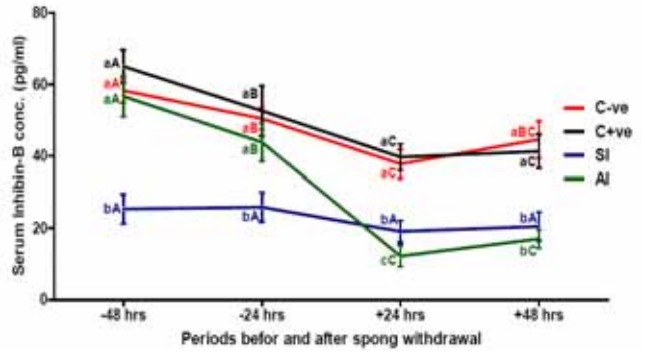

Figure 3: Serum Inhibin-B concentration (pg/ml) before 48 and 24 hrs.(A and B) and after 24 and 48 hr. (C and D) sponge withdrawal (at day 50 of treatment) in Awassi ewes injected, at 0, 28, and 50 days of treatment, with 4, 2 and 2 ml of normal saline (control; C-ve), 400, 200 and 200 µg of ovalbumine (positive control; C+ve), 400, 200 and 200 µg of synthetic inhibin (SI group), and 4 ml of normal saline followed by 4 and 2 ml of SFBFF antiserum (AI group). The values represented as M±SD. Different small letters denote significant difference ($p<0.05$) between groups for each period. Different capital letters denote significant difference ($p<0.05$) between periods for each group.

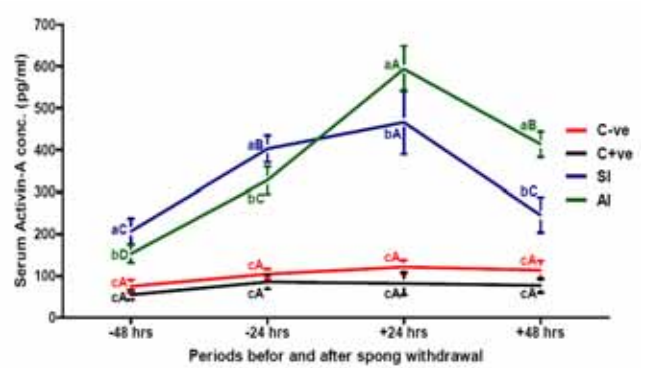

Figure 4: Serum Activin-A concentration (pg/ml) before 48 and 24 hrs.(A and B) and after 24 and 48 hr. (C and D) sponge withdrawal (at day 50 of treatment) in Awassi ewes injected, at 0, 28, and 50 days of treatment, 4, 2 and 2 ml of normal saline (control; C-ve), 400, 200 and 200 µg of ovalbumine (positive control; C+ve), 400, 200 and 200 µg of synthetic inhibin (SI group), and 4 ml of normal saline followed by 4 and 2 ml of SFBFF antiserum (AI group). The values represented as M±SD. Different small letters denote significant difference ($p<0.05$) between groups for each period. Different capital letters denote significant difference ($p<0.05$) between periods for each group.

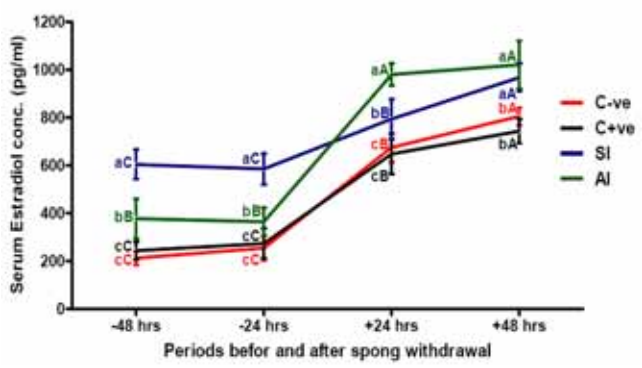

Figure 5: Serum Estradiol concentration (pg/ml) before 48 and 24 hrs. (A and B) and after 24 and 48 hr. (C and D) sponge withdrawal (at day 50 of treatment) in Awassi ewes injected, at 0, 28, and 50 days of treatment, 4, 2 and 2 ml of normal saline (control; C-ve), 400, 200 and 200 µg of ovalbumine (positive control; C+ve), 400, 200 and 200 µg of synthetic inhibin (SI group), and 4 ml of normal saline followed by 4 and 2 ml of SFBFF antiserum (AI group). The values represented as M±SD. Different small letters denote significant difference ($p<0.05$) between groups for each period. Different capital letters denote significant difference ($p<0.05$) between periods for each group.

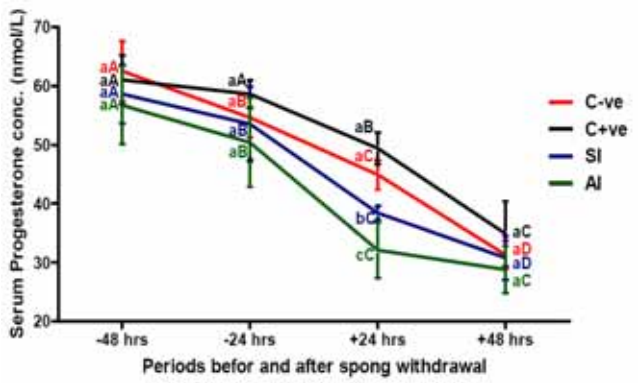

Figure 6: Serum Progesterone concentration (nmol/L) before 48 and 24 hrs. (A and B) and after 24 and 48 hr. (C and D) sponge withdrawal (at day 50 of treatment) in Awassi ewes injected, at 0, 28, and 50 days of treatment, 4, 2 and 2 ml of normal saline (control; C-ve), 400, 200 and 200 µg of ovalbumine (positive control; C+ve), 400, 200 and 200 µg of synthetic inhibin (SI group), and 4 ml of normal saline followed by 4 and 2 ml of SFBFF antiserum (AI group). The values represented as M±SD. Different small letters denote significant difference ($p<0.05$) between groups for each period. Different capital letters denote significant difference ($p<0.05$) between periods for each group.

Discussion

The present study pointed out that active and passive immunization against endogenous inhibin has potent role on reproductive hormone concentrations during the periods before and after sponge withdrawal in Awassi ewes. The decline of inhibin-B concentrations in active (SI) and passive (AI) immunized group ewes could be due to the immunoneutralization of endogenous inhibin subunits caused by generating of high level of the inhibin antibodies rapidly and steadily after booster immunizations (5,9,21-25), whereas it tend to return to the normal levels during and after 48hr of sponge withdrawal.

In both active and passive immunization, the current study reported significant elevation of serum activin-A concentration, which may attributed to the sharp decline of inhibins, where inhibins and activins are functionally antagonistic to each other (26). The inhibitory effect of inhibin on pituitary gonadotrophs was suppressed due to the immunoneutralization and subsequent decline of inhibin levels, these events along with the stimulatory effect of activin-A levels could be the main cause for FSH surge secretion from pituitary gonadotrophs. Thus the decrease in the levels of inhibin B in SI and AI treatment group ewes could induce the synthesis and secretion of a large amount of endogenous FSH from adenohypophesial gonadotrophs through the endocrine and autocrine/paracrine action of activin at the level of gonadotrope and granulosa cells (27,28). The autocrine/paracrine action of activin caused elevated FSHβ mRNA expression levels in the gonadotrophs of adenohypophesis leading to increased FSH biosynthesis and secretion (29).

Subsequent to the elevated secretion of FSH, ovarian follicular development could be increased, since FSH is the main stimulator of ovarian folliculogenesis (11). Follicular development is usually accompanied by granulose and theca cells proliferation. These changes are concomitant up regulation of the aromatase activity and subsequent increase in estradiol biosynthesis. This might explain the significant increase of serum estradiol concentration reported in the present study. The significant elevation of LH concentration after sponge withdrawal could be attributed to the elevated levels of estradiol through the positive feedback mechanism of estradiol on pituitary gonadotrophs (30,31). On the other hand, the role of activin, as a neuroendocrine reproductive controller, may be also act to modulate the LH-β biosynthesis and release which may be enhanced via modulation of LH-β subunit and GnRH receptor mRNA expression in response to activin action, which increased in the present results (32,33).

It can be concluded that the present elucidation change in reproductive hormones out of season breeding indicates a highly significant potency of both active and passive immunonutralization against endogenous inhibin in Awassi

ewes, and could play an important role in future animal reproduction applications.

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