

דוח מסכם לתכנית 07-216-820 בחינת הקשר בין דלקות עטין נמשכות תת-קליניות וקליניות
ושיבוש תפקודי רבייה בפרות חלב
מאת דוד וולפנזון וגבריאל לייטנר

הדוח כולל שלושה תקצירים של עבודות שהוצגו בשלושה כנסים בינלאומיים אירופאיים
בשנים 2006-7-8. לתקצירים הכתובים באנגלית צורפו ציורים וטבלאות מתאימים אשר מציגים את
הנתונים בהתאם.

ESDAR 2006

Paper submitted to:

European Society of Domestic Animal Reproduction

Clinical and subclinical mastitis effects on timing of ovulation, follicular and luteal growth and steroid concentrations in lactating cows

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Effects of naturally induced subclinical or clinical mastitis on ovarian functions were examined. Cyclic lactating Holstein cows (n=60) were diagnosed for mastitis type by somatic cells counts and bacteriological examination at the quarter level. Estrus synchronized cows were observed for estrus (E) and ovulation (O) times, and follicular and luteal growth were monitored by ultrasound. Steroids were determined in blood samples. Five of 20, and 6 of 25 cows with clinical and subclinical mastitis, respectively (~25%), manifested long E-O intervals, vs. only 1 of 15 healthy, control cows (7%; $P < 0.11$). The normal and long intervals were 28.5 ± 0.6 and 57.6 ± 10.4 h, respectively ($P < 0.01$). Size of preovulatory follicles was not affected by mastitis, but post-ovulation numbers of medium (6-9 mm) and large (≥ 10 mm) follicles were lowered by both types of mastitis ($P < 0.05$). Mid-cycle CL volume was smaller in the clinical mastitis group ($P < 0.06$). A 2.5-day delay in progesterone level rise during luteinization corresponded to delayed O in cows with long E-O interval. Concentrations of progesterone at mid-luteal phase and of cortisol around E were not affected by mastitis. Results show that 25% of cows infected by either subclinical or clinical mastitis manifested a long E-O interval that could disrupt fertilization. Both types of mastitis could alter follicular growth dynamics, but luteal and adrenal functions seemed to be unaltered.

BSAS 2007

Paper submitted to:

British Society of Animal Science

Mastitis induction of delayed ovulation and its relation to follicular functions and luteinizing hormone concentrations in lactating cows

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Introduction. Epidemiological studies have documented attenuated reproductive performance in cows having mastitis. Decreased conception and increased days to first AI as well as days open have been shown to be affected by both clinical and sub-clinical mastitis events, either before or after AI, induced by either gram-negative or gram-positive pathogens (Santos et al., 2004; Shrick et al., 2001). However, the actual reproductive mechanisms disrupted by pathogenic stress remain unclear. Exposure of cows to endotoxin (LPS) during the follicular phase reduced pulsatile luteinizing hormone (LH) concentrations and induced delayed ovulation (Suzuki et al., 2001). We showed (Lavon et al., 2004) that LPS administered at the onset of estrus induced delayed ovulation in 33% of cows. In naturally occurring mastitic cows, resumption of cyclicity post-partum has been shown to be delayed compared with that in healthy cows (Huszenicza et al., 2005). We recently found (Lavon et al., 2006), in cows synchronized with two doses of PGF2 α , that 26% (P<0.03) of cows with naturally occurring clinical or sub-clinical mastitis, compared with only 4% of healthy cows, manifested delayed ovulation, or did not ovulate. Delayed ovulation was reflected in a delay in the rise of progesterone. In addition, the duration of dominance of the preovulatory follicle tended to be longer, and the number of follicles during the cycle was 20% lower in mastitic than in healthy cows. The aim of the present study was to examine the effects of naturally occurring mastitis on the functioning of the preovulatory follicle and on gonadotropin secretion.

Methods. Cyclic lactating Holstein cows were diagnosed for mastitis type by somatic cell counts and bacteriological examination at the quarter level. Estrus-synchronized cows (95 days post-partum) were observed for estrus and ovulation times were determined by ultrasonography. Blood samples were taken for preovulatory estradiol and LH surge from 11 healthy cows, 10 cows that exhibited clinical mastitis events 33 days earlier, and 12 cows that exhibited sub-clinical, chronic mastitis. For pulsatile LH secretion, a 6-h window of frequent samples was taken from 5 healthy, 5 clinical mastitis, and 6 sub-clinical mastitis cows during the follicular phase 36 h after PGF2 α . Hormones were determined by validated RIA or ELISA. Data were analyzed by ANOVA using the GLM of SAS. Means and SE are presented.

Results. Cows with clinical or sub-clinical mastitis, which manifested an extended estrus-to-ovulation interval, exhibited lower concentrations of estradiol at estrus, compared with healthy cows or mastitic cows, which manifested normal estrus-to-

ovulation intervals (3.3 ± 0.4 , 5.8 ± 0.5 , and 5.9 ± 0.8 pg/ml, respectively; $P<0.01$). Healthy cows or mastitic cows (clinical or sub-clinical) with normal intervals, exhibited normal peaks of LH surges at the expected time relative to onset of estrus (6.9 ± 0.6 ng/ml, 4.6 ± 0.6 h). In contrast, mastitic cows with delayed ovulation exhibited a delayed LH surge (10.5 ± 3 h relative to onset of estrus, $P<0.01$) with either low or normal LH surge, or no LH surge was evident. Regarding pulsatile LH, no statistical differences were detected between healthy, clinical, or sub-clinical mastitic cows in their mean, pulse amplitude, and pulse frequency of LH concentrations (1.3 ± 0.1 ng/ml, 0.73 ± 0.1 ng/ml, 4.9 ± 0.2 pulses/6 h, respectively).

Conclusions. Results indicate that short-term clinical mastitis events or sub-clinical, chronic mastitis induced, in about 30% of the cows, a delayed ovulation syndrome associated with low estradiol concentrations. However, this was not associated with altered pulsatile LH secretion during the follicular phase, suggesting a direct effect of mastitis on steroidogenesis. Delayed ovulation, which was associated with low or delayed LH surge, could be related to low preovulatory estradiol levels. The above could explain, in part, mastitis-induction of low fertility in dairy cows.

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ICAR 2008

Paper submitted to:

International Congress of Animal Reproduction

Subclinical mastitis lowers steroid concentrations and gene expression in preovulatory follicles of lactating cows

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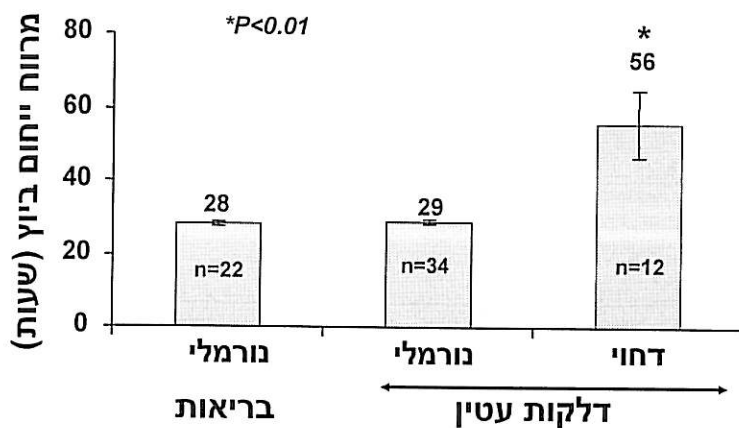
Introduction. Recently we showed that 30% of cows with naturally occurring mastitis had low estradiol levels at estrus and low or delayed LH surge, resulting in delayed ovulation. These data suggest that mastitis may directly affect ovarian follicles. The aim of this study was to examine the effect of mastitis on the characteristics of the preovulatory follicle in cows.

Methods. Cyclic lactating Holstein cows were diagnosed for mastitis by somatic cell counts and bacteriological examinations. Follicular fluids and granulosa cells were aspirated from preovulatory follicles of synchronized cows by ultrasound-guided procedure. Total RNA was isolated from granulosa cells and gene expression was determined by real-time quantitative PCR. Data were analyzed by ANOVA, and means and SE are presented.

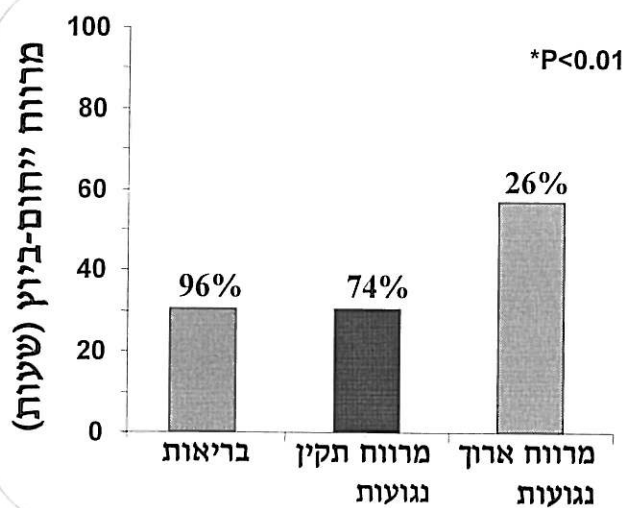
Results. Of 16 cows with sub-clinical mastitis, about 30% (n=5) exhibited low estradiol concentrations in the follicular fluid, as compared with 70% (n=11) exhibiting normal estradiol levels (42±19 vs. 654±90 ng/ml, respectively; P<0.01). Cows with sub-clinical mastitis and low follicular estradiol levels also exhibited low plasma estradiol concentrations. Such a distribution in estradiol levels was not observed in healthy cows (n=17) or in those exhibiting a clinical mastitic event (n=8) about 43 days earlier (692±101 and 1026±177 ng/ml, respectively). Similarly, the follicular androstenedione levels were about 9-fold lower (P<0.05) in the cows with sub-clinical mastitis and low estradiol levels, compared with the three other groups. Progesterone levels did not differ among groups. Cows with sub-clinical mastitis exhibiting low estradiol concentrations (n=4) also had a 5 to 8-fold reduction in mRNA expression for P-450-aromatase and LH receptors (LHr), compared with healthy (n=10) and clinical mastitic (n=4) cows (P<0.05); a 4-fold reduction (P<0.05) in LHr, but not in the expression of P-450-aromatase, was noted in comparison to cows with sub-clinical mastitis exhibiting normal estradiol levels (n=6). Expression of mRNA for FSHr and inhibin did not differ among groups.

Conclusions. These findings suggest that in 30%, the presence of sub-clinical mastitis induced a pronounced decrease in steroid concentrations and gene expressions in preovulatory follicles. Reduced gene expression, resulting in low follicular steroid concentrations and low preovulatory plasma estradiol levels could consequently delay LH surge and ovulation, as documented in our earlier studies. These mechanisms may explain, at least partially, mastitis-induced low fertility in dairy cows.

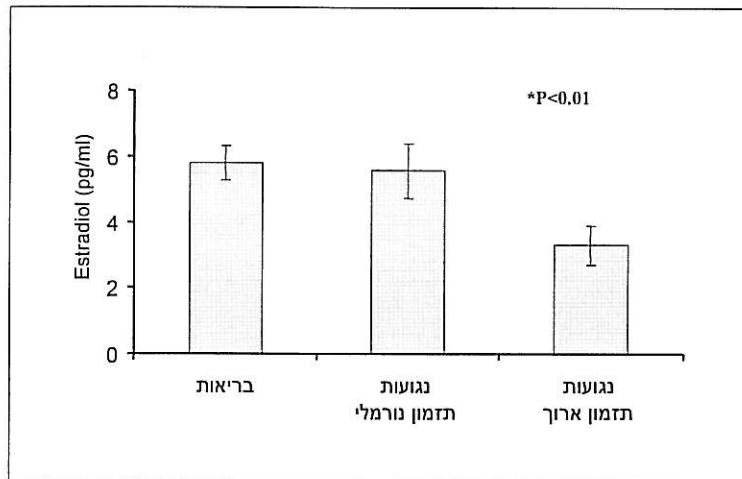
התארכות מרווח הזמן בין תחילת הייחוס
 לבין הביוץ נמצאה ב- 30% הפרות



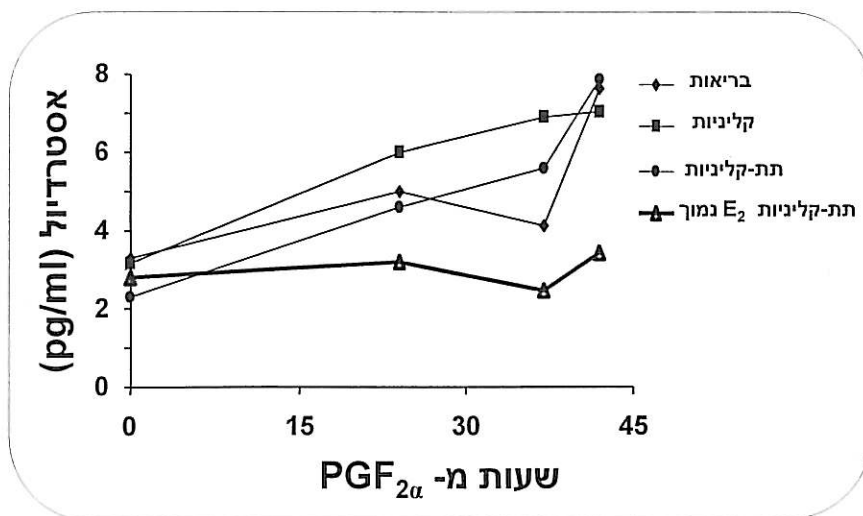
התארכות מרווח הזמן בין תחילת הייחוס לבין מועד הביוץ
 ברבע מן הפרות



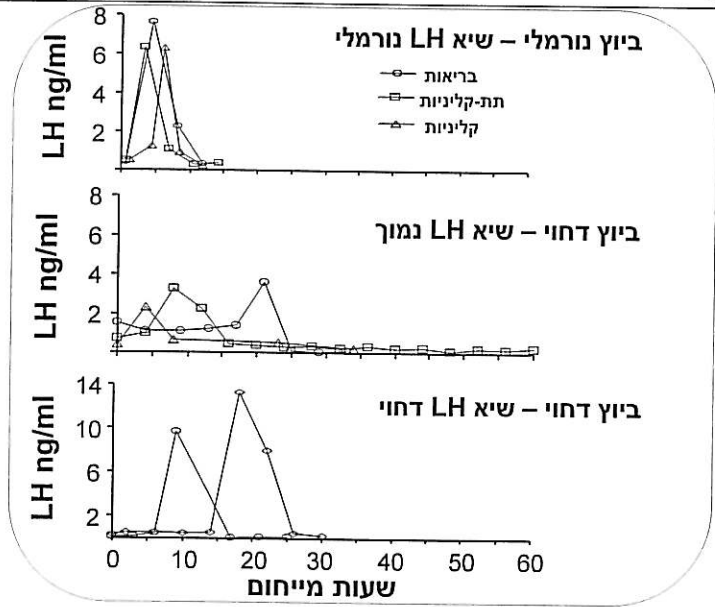
ריכוז אסטרוגן בייחוס יורד משמעותית בפרות נאצות (קלינית-קלינית) שהצילו ביוץ דחוי בהשוואה לבריאות או נאצות עם תזמון נורמלי



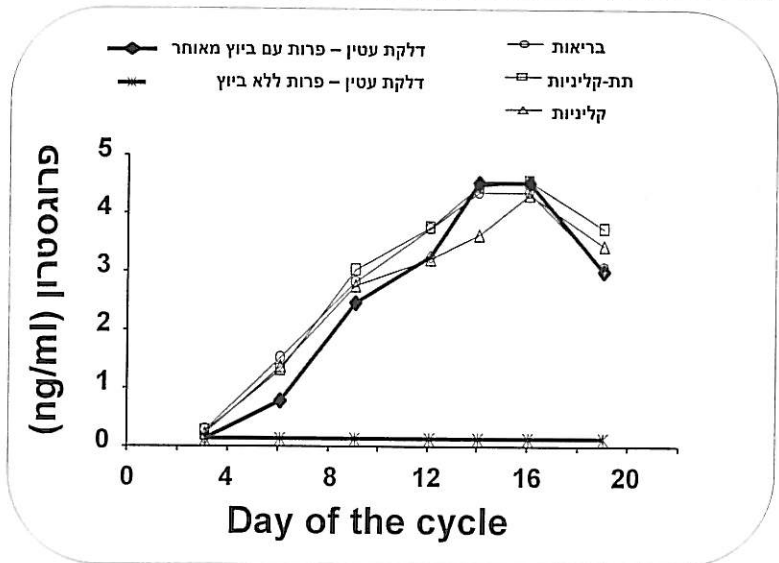
ריכוזי אסטרוגן בפלסמה של הפוליקולרי של המחזור נמוכים משמעותית בהפרות הנאצות



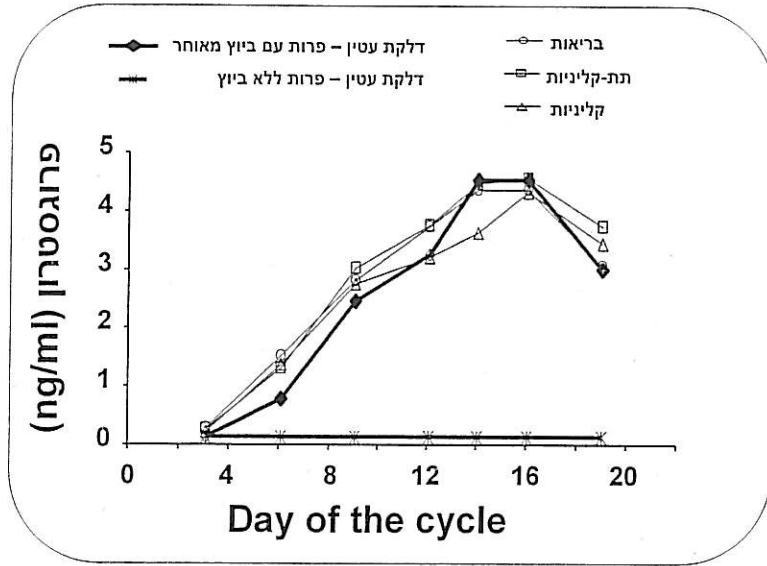
**ראות שיא LH נמוכות או דחיות
נמצאו ב 30% מהפרות המציינות ביוץ דחוי**



**התאמה, דחיה בהתפתחות אוליגוזואידים
(צפיית פרוגסטרון) הפרות שהציינו ביוץ דחוי**



**בהתאמה, דחייה להתפתחות אולי 3הוה
(עליית פרוגסטרון) בפרות שהציאו ביוץ דחוי**



לא נמצאה השפעה עקיפה של ירידת הפרשת אונדורופוניס על תיפקודי הזקיב בפרות עם דלקת עטין תת קלינית או קלינית שהסתיימה לאחרונה

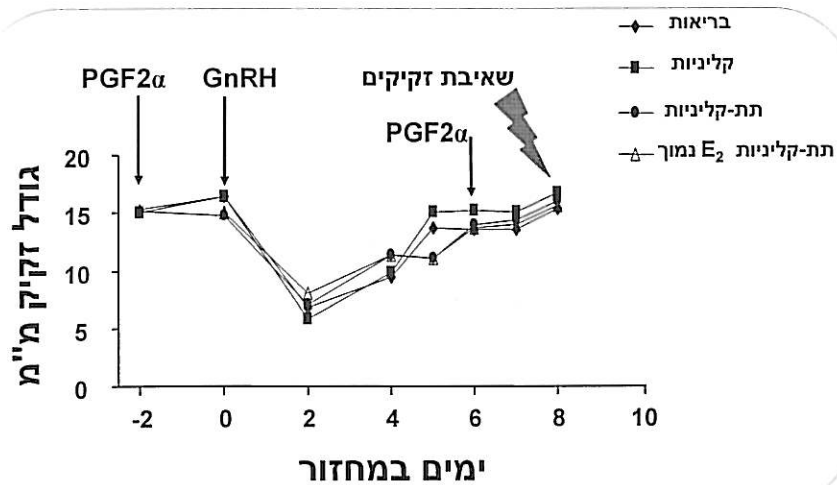
ההפרשה הפואסטיבית של LH בפאזה הפואיקולארית לא השתנתה בפרות נאוצות

אמפלוטודה	פואסטיב' e	ממוצע	
0.84	4.6	1.3	בריאות
0.69	5.3	1.2	תת קליני
0.67	4.8	1.4	קליני

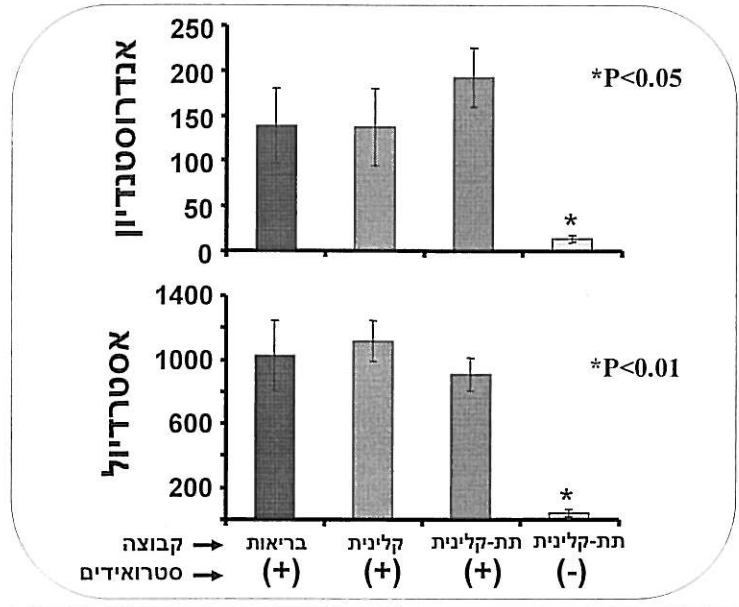
סיווג הפרות לפי סוג הדלקת, סוג החיידק ואיכות הזקיק.

	דלקות תת-קליניות		דלקות קליניות	
	תקין	לא תקין	תקין	לא תקין
CNS	5	4		
Strep sp.	2	1		
E. Coli (chronic)	3			
Proteos	1			
E. Coli			8	1

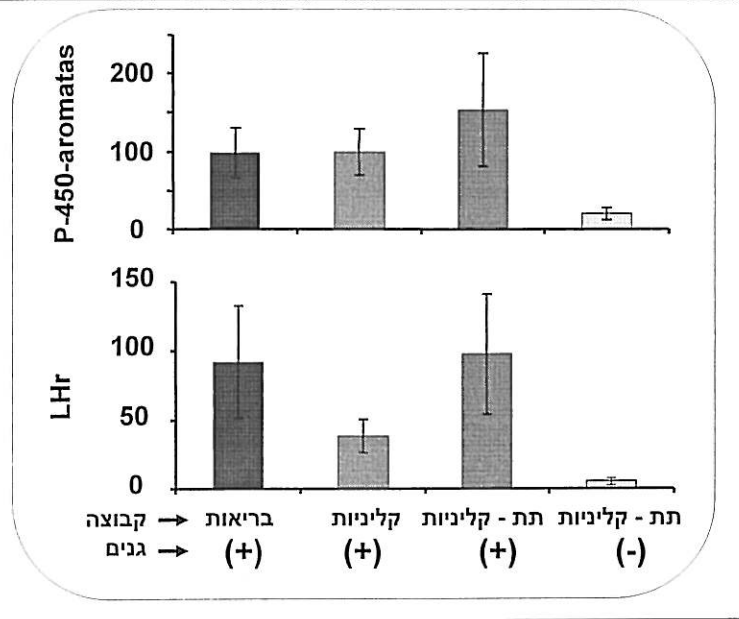
אודף הזקיק הפראופולטורי אינו שונה בין הקבוצות



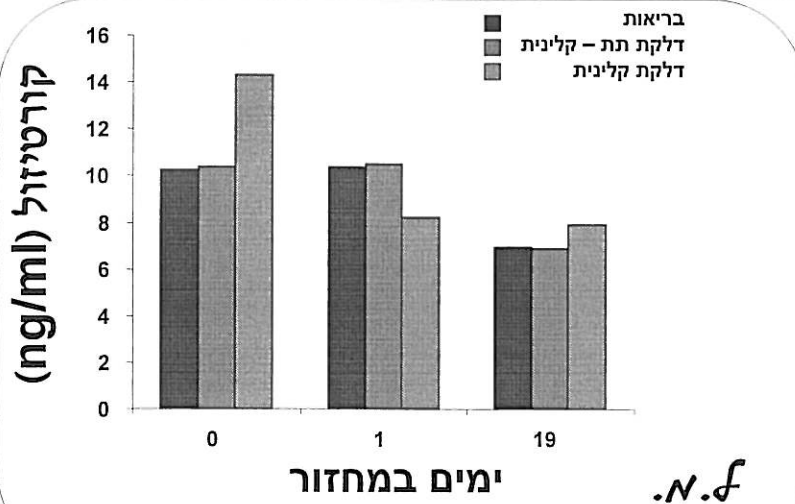
שליש מהפרות הנאוצות בדלקת תת-קלינית מציאות
ריכוז נמוך של סטרואידים בנוזל הזקיק (ng/ml)



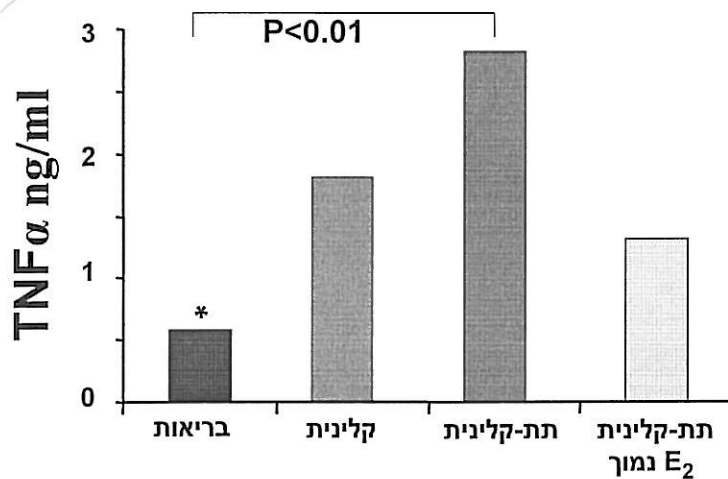
שליש מהפרות הנאוצות בדלקת תת-קלינית מציאות
ירידה בהתבטאות הגנים לארומטאז ול LHR (AU)



ריכוזי קורטיזול אינם שונים בפרות נאוצות
 בדלקות עטין כרוניות ולאכן אינם יכולים
 להסביר ירידה בתיפקודי הלתיקי



ריכוזי $TNF\alpha$ במסמה אופיט אפחות נאוצות
 בדלקות עטין אק לא מסבירים את הפסיא שאר
 הלתיקי הפיט אפחות הנאוצות



סיכום

1. דלקת צטין תת-קלינית אורמת ב-30% מהפרות (מסיבה שאינה ברורה) לפגיעה באיכות הזקיק המבויץ.
2. פגיעה זו באה לביטוי בירידת ריכוזי סטראידים בפלסמה ובנוזלים פוליפיקולריים, ובהתבטאות נמוכה של אנטי הקשורים ליוזר סטראידים.
3. ממצאים אלו מצידים על פגיעה ישירה של דלקות צטין תת-קליניות בזקיקים בשחלה.
4. הממצאים מסבירים חלק מהסיבות לפגיעה בפוריות.

מסקנה

הדחייה של הביוץ בכ 30% מהפרות הנלוות בדלקת צטין היא ברת תיקון באמצעים הורמונליים. תכנית מחקר נמשכת חדשה שלנו, המבוססת על ממצאי התכנית הנוכחית, תבחן שיפור התוצאות פרות נלוות באמצעים הורמונליים או משקיים אחרים.