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Thymic proliferative response during different physiological states: a comparative study

* Habbal O A[1], McLean I M[2], Abu-Hijleh M F[3]

الاستجابة التكاثرية لخلايا الغدة السعترية (الثيموسية) في حالات وظيفية (فسيولوجية) مختلفة: دراسة مقارنة

ع. الحبّال ، ج. ما كلين ، م. أبو حجله

الملخص: الهدف: دراسة الاستجابة التكاثرية لخلايا الغدة السعترية (الثيموسية) خلال حالات فسيولوجية مختلفة وذلك للتمييز بين التغيرات التي تحدث نتيجة إفراز هرمونا ت الستيرويد عن تلك الناتجة بسبب وجود الحيوانات المنوية أو الجنين المبكر في الجهاز التناسلي للأنثى.
الطريقة: تمت متابعة التغيرات يومياً خلال فترات الطمث والحمل الكاذب والحمل المتماثل جينياً ، وذلك باستخدام جرذان ناضجة من فصيلة (RT1^u) AO . ولقد احتوت كل مجموعة يومية على 6 جرذان على الأقل. **النتائج:** لوحظ أن هناك ارتفاعا متكرراً في الاستجابة التكاثرية لخلايا الغدة السعترية في اليوم الثاني لفترة الطمث ، والذي لربما كان لتهيئ الأنثى للتحديات المناعية الناتجة عن الجماع. ويمكن إرجاع ذلك إلى وجود هرمون الأستروجين المعروف بتواجده بصورة مرتفعة خلال هذه الفترة. ومع استحثاث الحمل الكاذب ، تم الحصول على نتائج مماثلة لاستجابة الغدة السعترية خلال اليوم الثالث والذي يتصادف مع الارتفاع المعروف عنه في إفرازات الإستروجين خلال هذه الفترة. ومع حدوث الحمل المتماثل جينياً ، لوحظ حدوث هبوط مناعي ملحوظ خلال اليوم الثالث والذي تبعه إرتفاع ملحوظ خلال اليوم الخامس مقارنة بنفس الفترة للحمل الكاذب. **الخلاصة:** تبعاً لذلك فإنه من المعتقد أن الهبوط المبدأي في الاستجابة التكاثرية لخلايا الغدة السعترية يمكن أن يكون نتيجة الخواص المناعية المحبطة للسائل المنوي وذلك بقصد حماية الجنين المبكر خلال فترة ما قبل الغرس في الرحم. وأما الزيادة الملحوظة في الإستجابة التكاثرية لخلايا الغدة ، فإنه من المقترح بأنها إستجابة مناعية لعملية الغرس الجيني نفسها.

ABSTRACT: ***Objective*** –To study the thymic proliferative response during different physiological states to distinguish those changes due to alterations in steroid hormone secretion from those resulting from the presence of spermatozoa and/or early conceptual products in the female reproductive tract. ***Method –*** Using mature female rats of an inbred AO(RT1^u) strain, observations on the thymus were made at 24 hour intervals during the oestrous cycle, early pseudopregnancy and early syngeneic pregnancy. Each daily group contained a minimum of 6 animals. ***Results*** – During the oestrous cycle, a significant mid-cycle increase of thymocyte proliferation occurred during dioestrus which peaked on day 2, and as a repetitive response may be a preparation for a coital challenge. This response may be oestrogen-dependent since oestrogen levels begin to increase during early dioestrus. The induction of pseudopregnancy generates a comparable but delayed increase in thymic proliferative activity. Since thymocyte proliferation and oestrogen secretion both peak on day 3 of pseudopregnancy, such a response may indeed also be oestrogen-dependent. After syngeneic mating, there was a significant depression in thymic proliferative activity on day 3 followed by a significant increase on day 5 compared with the same days of pseudopregnancy. ***Conclusion*** – This initial depression of proliferative activity may be induced by the immunosuppressive action of seminal plasma, to safeguard the preimplantation conceptus while the day 5 increase in cellular proliferation suggests a response to implantation.

KEY WORDS: oestrous cycle, pseudopregnancy, implantation, seminal fluid, thymus

For a number of years it has been apparent that manipulations of the maternal immune system can have both positive and negative effects on fertility. For example, abortion can be induced by immunisation of experimental animals with sperm[1] or tumour cells,[2] whereas recurrent abortions in certain strain combinations of mice can be dramatically reduced by immunisation with cells carrying paternal major histocompatibility complex (MHC).[3] Striking thymic changes are evident after adrenalectomy[4] and gonadectomy[5] in murine laboratory animals. The presence of high affinity oestrogen receptors, confined to thymic epithelial cells indicates that the thymus is a target organ for oestrogen.[6] Dynamic remodelling of its structure occurs after oestradiol treatment[7], and varying oestrogen levels can alter thymic hormone

[1]Department of Anatomy, College of Medicine, Sultan Qaboos University, P.O.Box: 35, Postal Code: 123, Muscat, Sultanate of Oman.
[2]Department of Cell and Structural Biology, Division of Anatomy, Medical School, University of Manchester, Manchester, U.K.
[3]Department of Anatomy, Faculty of Medicine, Jordan University of Science & Technology, Irbid, Jordan.

*To whom correspondence should be addressed.

output.[8] Serum levels of progesterone vary during the ovarian cycle but progesterone is apparently the only hormone essential for the maintenance of pregnancy in every species studied.[9] However, the effects of sex steroids on immunity and the lymphoid tissues remain controversial.[10]

Sterile coitus and experimental stimulation of the uterine cervix induce a state of pseudopregnancy in which the oestrous cycle is interrupted and a di-oestrous state persists as confirmed by daily vaginal smears. The classical response of the early pseudo-pregnant uterus to an appropriate stimulus is the formation of deciduomata.[12] These are well-defined areas of endometrial reaction made up of decidual cells similar to those occurring during early pregnancy, and this response cannot be elicited during the normal oestrous cycle. During pseudopregnancy, progesterone level reaches that of the pro-oestrous phase by the fourth or sixth day[13] and are sustained at that level until the ninth or tenth day.[14] Thereafter, the progesterone level falls steadily until the twelfth day.[15] Although progesterone priming alone is sufficient to induce the cellular changes associated with the decidual reaction, the addition of oestrogen influences the uterine response, since it increases the size of the deciduoma and regulates the length of time during which the stimulus is effective in rats.[16] Serum assays of oestrogen during pseudopregnancy indicate a surge on the third and fourth day.[17] There is considerable evidence to suggest that spermatozoa possess surface alloantigens,[18,19] whereas secretions from the prostate and other parts of the male reproductive tract probably constitute another antigen system.[20] During the first 5 days of pregnancy, the female reproductive tract is exposed to an ejaculate of spermatozoa in seminal fluid and a number of preimplantation embryos. In the latter part of this preimplantation period, the embryo sheds its zona pellucida preparatory to implantation. Since the conceptus expresses paternal alloantigens,[21] any immune response, particularly a cell-mediated one, generated by non-fertilising spermatozoa could hazard the survival of the preimplantation embryo. The zona pellucida provides the initial protection, but with its dissolution, some other mechanism must operate to safeguard the implanting conceptus from immunological attack.

Central to the allograft reaction is cell proliferation within the T-cell population of the regional lymph nodes, where it is associated with weight gain.[22] In the rat, the regional lymph node response to subcutaneous challenge with spermatozoa is unequivocal[23] and includes the generation of specific anti-male strain cytolytic T-cells.[24] In addition to its role as the suspensory agent for spermatozoa, seminal fluid contains a number of immunosuppressive factors which may protect spermatozoa from immunological damage and prevent sensitization of the female to spermatozoal antigens following coitus.[20] Seminal fluid can interfere directly or indirectly with the function of T-cells,[25] B-cells,[26] natural killer cells (NK cells),[27] antibody[28] and complement.[29] It also affects cell-mediated immunity as it limits the duration of an effective cytolytic T-cell response to ejaculated spermatozoa.[30] The ejaculate therefore contains highly immunogenic cells and a number of factors, which appear capable of modifying an immune response.

The response of the thymus during pregnancy is generally considered one of involution. In the mouse, observations are of continuous involution from the onset of pregnancy[31] or involution beginning later during pregnancy.[32] In the rat, this variability in the onset of thymic involution during pregnancy has been shown to be strain-dependent[33] and this presumably explains the conflicting results found in mice. The gestational thymus of the rat has also been observed to be associated with increased levels of thymocyte proliferation following allogeneic pregnancy.[33] Such an immunological effect upon the thymus is also demonstrable in analyses of thymic responses during syngeneic and allogeneic pregnancy in mice[34] and rats.[35] These changes in the thymus associated with pregnancy are presumably the result of hormonal changes as well as the presence of the ejaculate and the products of conception. Neonatal thymectomy in mice delays vaginal opening, an effect that may be due to a thymic-ovarian interaction.[36]

This study primarily collates and compares the changes in the thymus during the oestrous cycle, early pseudopregnancy and early syngeneic pregnancy. The study was done in an attempt to distinguish those changes induced by the presence of spermatozoa and/or the early zygote from those occurring as a result of alterations in levels of steroid hormone secretions. The authors' previous work[35,37] looked broadly into the lymphoid tissue changes that occur during each of the three hormonal states.

METHODS

THE ANIMALS

A highly inbred strain of rat, AO(RT1^{U}/AgB2), was used in this investigation. The sexually mature female animals were bred from a specially maintained stock fed on a standard diet with a 12 hours light cycle. At the time of sacrifice, the rats had body weights of 160–200 g, and were between 13 and 15 weeks of age.

The latter choice minimised age-related changes in the lymphoid tissues.

DETERMINATION OF OESTROUS CYCLE STAGE

In the sexually mature rat, the various stages of the oestrous cycle were determined by low-power (×40) microscopic observation of the unstained smear taken daily with a wire loop from the lower vagina.[38] Although the rat cycle is subject to individual variations, the oestrous phase recurs every 4–5 days in the absence of mating.[39] In the present study, vaginal smears were prepared from the virgin rats at 10.00 hrs each day. The oestrous phase animals, designated group 0, were sacrificed at approximately 11.00 hrs on the day oestrus was identified in the vaginal smear. The remaining groups, designated 1–4, were sacrificed at 24–hour intervals, when the vaginal smears confirmed the appropriate phase of the cycle. All groups contained at least 6 animals each and these were sacrificed without cervical stimulation or mating over the period of the oestrous cycle.

INDUCTION OF PSEUDOPREGNANCY

Pseudopregnancy was induced in virgin rats by mechanical cervical stimulation during oestrus. An electrical vibrator was used for this purpose and the stimulation was maintained for a minimum of 30 seconds in the restrained animal.[40] This method was chosen because of its simplicity and effectiveness.[40] The day following cervical stimulation was designated the first day of pseudopregnancy. Groups of animals, designated 1–5, were sacrificed at 24–hour intervals as successive daily smears confirmed the presence of a dioestrous-like vaginal smear characteristic of pseudopregnancy.

MATED ANIMALS

In the mated (pregnant) animals, 1 to 3 virgin female rats in oestrus were caged late in the afternoon with a male rat of proven fertility. Vaginal smears were prepared from the rats at 10.00 hrs each day. The day spermatozoa were noted in the smear was designated day 1 of pregnancy. The remaining groups designated days 2 to 5 were sacrificed at 24-hour intervals following day 1 of pregnancy.

REMOVAL OF THE THYMUS AND PREPARATION OF CELL SUSPENSIONS

On the day of sacrifice, all the animals were sacrificed at approximately 11.00 hrs after verification of the vaginal smear. Each animal was anaesthetised with ether and subsequently given an i.p. injection of 12 mg of veterinary nembutal ('Sagatal': May & Baker Ltd., Dagenham). The weight of the animal was recorded and after exsanguination, the thymus was removed and cleaned, weighed and placed in 199 tissue culture medium (Wellcome Reagents Ltd., Beckenham) adjusted to pH 7.2. A cell suspension from each thymus was prepared by repeated squashing with a plastic pestle. This cell suspension was subsequently filtered through a stainless steel mesh (144 holes/cm^2) and made up to a final volume of 10 ml. From this, 1 ml was taken and used for cell counting, with a Coulter ZB counting instrument.

RADIOLABELLING AND PREPARATION OF CELL SMEARS

After removal of aliquots for the cell counts, 10 µl of [6–^{3}H] thymidine (Amersham International plc., Aylesbury) at an activity of 100 µCi/ml were added for each 1 ml of cell suspension. After mixing, the suspensions were incubated in a water bath at 37°C for 1 hour with occasional shaking. The suspensions were then centrifuged at 2000 rpm (700 g) for 10 minutes and the resultant pellets resuspended in phosphate buffered saline (PBS) at a concentration of 5×10^6 cells/ml. Previous work has shown that at this dilution the cell suspension requires no further washing. This cell concentration is also optimal for preparing smears in the Shandon-Elliot cytocentrifuge. After radiolabelling, six smears were prepared from each thymocyte suspension. The smears were prepared on slides previously coated with gelatin/chrome alum[41] to prevent peeling of the nuclear emulsion used in the autoradiography. The smears were dried in air and fixed in methanol, dipped in diluted Ilford K2 nuclear emulsion (Ilford Ltd., Knutsford) and stored at 4°C in the dark for three weeks. After this period, when a trial slide showed clear labelling, the remaining slides were developed in Kodak D19 developer, fixed in acid-hardening fixer, stained with haematoxylin and eosin and mounted.

RADIOLABELLED CELL COUNTS

A non-random strip counting method was used to assay, at ×400 magnification, the number of labelled and unlabelled cells and thus the percentage of cells labelled. Total labelled cell counts were then calculated from the total thymocyte counts. Only cells in the DNA-synthesis 'S' phase of mitosis incorporate thymidine.[42] Assuming a random distribution of mitotic phases, a constant proportion of the actively proliferating cells, approaching 50%,[43] will be in the 'S' phase. The consistency and low standard errors of the results obtained from each group in this study support this assumption. Consequently, it seemed reasonable to use the figures for percentage labelling and total labelled cell counts as a measure of proliferative activity; not an absolute measure but an accurate reflection of the relative differences between groups.

Statistical Analysis

The data were analysed in two ways. Comparisons were made between the different time points of animals in the same hormonal state and comparisons were made between the same time point, of animals in different hormonal states. Where a one-factor analysis of variance gave a significant result, this was further analysed by a comparison of individual group means using the least significant difference method. Two group means differing by more than the least significant difference value indicates a significant difference at the 0.05 level or less.

RESULTS

In this study, a number of observations were made on the thymus. These observations were recorded at 24-hour intervals throughout the oestrous cycle and during the first five days of pseudopregnancy and syngeneic pregnancy (tables 1–3). The time interval between the observations and the limitation of the observation period allowed the results obtained on different days in each of the three physiological states, and those obtained on comparable days in any two states, to be compared. The observations made on oestrous phase virgin animals, day 0 of the oestrous cycle, provided base-line data for the pseudopregnant and pregnant animals since the induction of pseudopregnancy and the initiation of pregnancy took place on that day. The results obtained during days 1–4 of pseudopregnancy and pregnancy were compared with those of days 1–4 of the oestrous cycle while the day 5 results from the stimulated animals were considered comparable with the oestrous phase results of the unstimulated animals since in such animals the oestrous phase recurs every fifth day. Each group contained at least 6 animals and each animal yielded figures for body weight, thymic weight, total thymocyte content both absolute and relative to thymic weight, percentage of labelled cells and total labelled cell counts.

Although body weight rose to a maximum value on day 2 of the oestrous cycle, it had returned to its oestrous phase value on day 4. No significant changes were observed during either of the other two physiological states or between any of the three states. No significant changes in thymic weight were observed during the oestrous cycle, early pseudopregnancy or early syngeneic pregnancy. However, the thymus was significantly heavier on day 4 of pseudopregnancy and syngeneic pregnancy than on the comparable day of the oestrous cycle.

Observations of the total thymocyte counts (figure 1) within each of the three physiological states again showed no significant differences. However, on comparing these physiological states, the total thymocyte counts on day 4 of pseudopregnancy and days 2, 3 and 4 of syngeneic pregnancy, were significantly greater than those on the comparable days of the oestrous cycle, while the count on day 2 of syngeneic pregnancy was significantly greater than that on the same day of pseudopregnancy. The only significant differences in thymic cell densities (figure 2)

TABLES INDICATING THE MEANS OF THYMIC RESULTS

Table 1

Oestrous Cycle

Day of Oestrous Cycle		0	1	2	3	4	5
Thymic weight		292.00	252.00	282.00	268.00	227.00	292.00
	+/-	*14.14*	*18.93*	*18.70*	*11.81*	*18.04*	*14.14*
Total thymocyte content x10^6		893.00	779.00	759.00	785.00	682.00	893.00
	+/-	*53.50*	*89.23*	*75.14*	*64.01*	*70.72*	*53.50*
Cell density x 10^6/100 mg tissue		308.00	304.00	269.00	291.00	299.00	308.00
	+/-	*22.59*	*13.13*	*18.59*	*17.69*	*14.78*	*22.59*
% Cell proliferation (size)		8.44	7.65	12.22	11.14	7.65	8.44
	+/-	*0.70*	*0.18*	*0.78*	*0.62*	*0.48*	*0.70*
% labelled (proliferating) cells		8.23	6.27	13.17	10.50	6.24	8.23
	+/-	*1.14*	*0.43*	*1.84*	*0.56*	*0.78*	*1.14*
Total labelled cells x 10^6		74.34	48.13	95.44	81.95	40.55	74.34
	+/-	*13.10*	*5.42*	*13.67*	*7.04*	*2.95*	*13.10*

TABLE 2

Pseudopregnancy

Day of pseudopregnancy		0	1	2	3	4	5
Thymic weight mg		293.00	397.00	273.00	262.00	275.00	279.00
	+/-	*14.14*	*39.68*	*18.46*	*15.00*	*22.03*	*8.71*
Total thymocyte content x10^6		893.00	962.00	816.00	931.00	955.00	903.00
	+/-	*53.50*	*125.83*	*65.65*	*69.66*	*133.47*	*35.86*
Cell density x 10^6/100 mg tissue		308.00	320.00	297.00	329.00	339.00	334.00
	+/-	*22.59*	*20.23*	*9.39*	*16.68*	*21.72*	*9.85*
% Cell proliferation (size)		8.44	9.39	10.67	12.96	8.56	7.36
	+/-	*0.70*	*0.55*	*0.54*	*1.66*	*0.51*	*0.17*
% labelled (proliferating) cells		8.23	7.04	8.96	12.82	8.88	5.19
	+/-	*1.14*	*0.47*	*0.87*	*0.85*	*1.16*	*1.28*
Total labelled cells x 10^6		74.34	56.89	71.48	118.31	80.12	44.74
	+/-	*13.10*	*7.01*	*7.17*	*10.04*	*10.31*	*9.28*

TABLE 3

Syngeneic pregnancy

Day of syngenic pregnancy		0	1	2	3	4	5
Thymic weight		292.00	281.00	289.00	298.00	311.00	291.00
	+/-	*14.14*	*21.25*	*10.54*	*10.57*	*16.57*	*21.99*
Total thymocyte content x10^6		893.00	961.00	1008.00	942.00	973.00	977.00
	+/-	*53.50*	*98.04*	*64.37*	*61.89*	*63.64*	*99.26*
Cell density x 10^6/100 mg tissue		308.00	339.00	349.00	315.00	312.00	333.00
	+/-	*22.59*	*14.33*	*15.13*	*11.92*	*8.06*	*14.98*
% Cell proliferation (size)		8.44	7.47	7.84	10.24	9.58	7.36
	+/-	*0.70*	*0.22*	*0.58*	*0.21*	*0.61*	*0.19*
% labelled (proliferating) cells		8.23	7.18	7.64	10.80	10.92	8.80
	+/-	*1.14*	*0.45*	*0.40*	*0.64*	*0.75*	*0.31*
Total labelled cells x 10^6		74.34	70.60	77.56	100.63	105.70	86.45
	+/-	*13.10*	*10.96*	*7.59*	*6.04*	*9.09*	*10.06*

wererecorded on day 2 when the density observed during syngeneic pregnancy was significantly greater than that on the same day of pseudopregnancy and the oestrous cycle.

During the oestrous cycle, thymocyte proliferation (figure 3) peaked on day 2 and was significantly greater on days 2 and 3 than on day 0 (oestrus), 1 and 4. After the induction of pseudopregnancy, proliferative activity was significantly greater on day 3 than on any other day, and on days 2 and 4 was significantly greater than that on day 5. Following syngeneic mating, thymocyte proliferation was significantly greater on days 3 and 4 than on post-coital days 1 and 2. When comparing thymocyte proliferation during the oestrous cycle with that during pseudopregnancy, there was significantly greater mitotic activity on days 3 and 4 of pseudopregnancy than on the same days of the oestrous cycle. When comparing the levels of thymocyte proliferation during the oestrous cycle with those after syngeneic mating, the post-coital level on day 4 was significantly greater than that on the comparable day of the oestrous cycle. A comparison of the results obtained during pseudopregnancy with those after syngeneic mating demonstrated a significantly greater level of thymocyte proliferation on the fifth post-coital day than on the same day of pseudopregnancy.

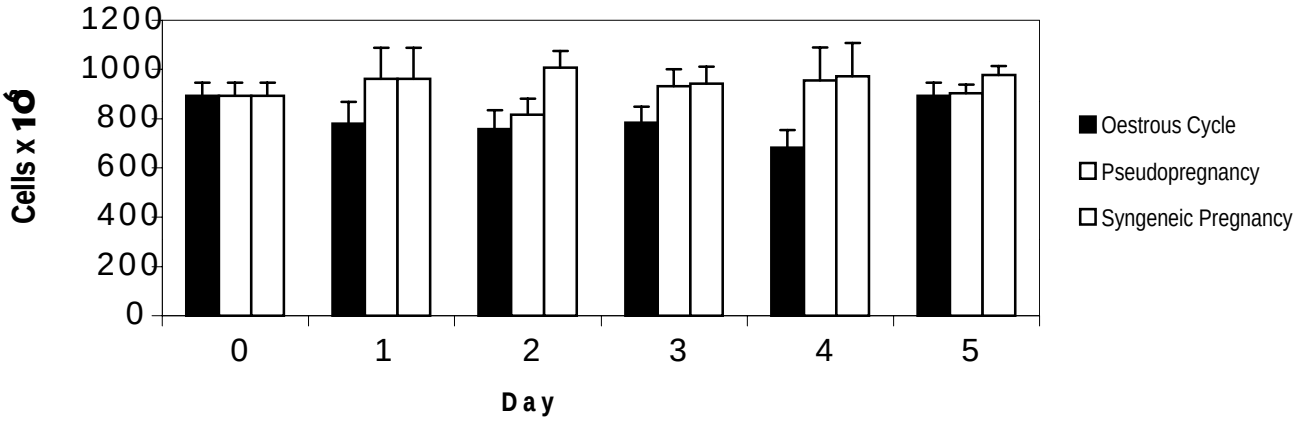

FIGURE 1. *Total thymocyte counts*

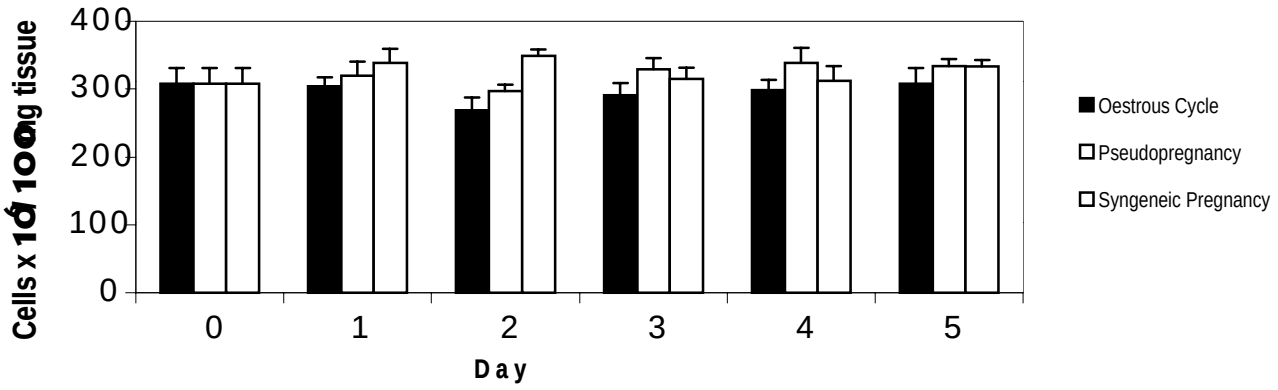

FIGURE 2. *Thymic cell density*

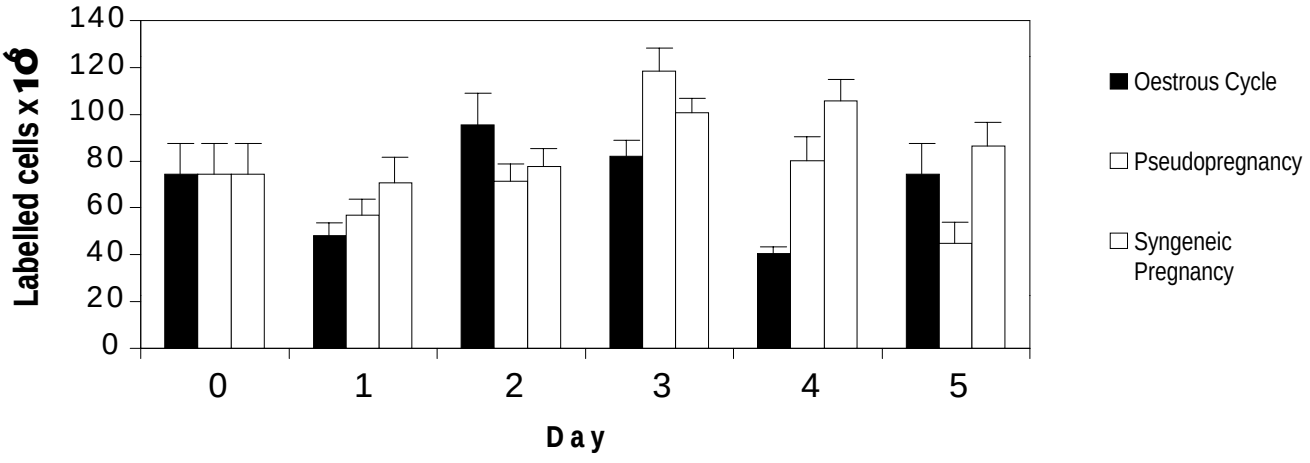

FIGURE 3. *Total proliferating thymocyte counts*

DISCUSSION

The thymus is a primary lymphoid organ and its central role in the immune system, as the source of T cells, was demonstrated over 30 years ago.[44] Recent research suggests that all immunoregulatory processes are part of an integrated response involving neural, endocrinal and immune systems.[45] Given the central role of the thymus in the immune system and the complex neuroendocrinological events involved in the initiation and continuance of pregnancy in eutherian mammals, it is not surprising that the gestational thymus has been the focus of much research[46] concerning the immunological paradox of pregnancy. This study, however, had more limited objectives and was confined to observations on the rat thymus during the oestrous cycle and for five days following interruption of the oestrous cycle by either mechanically induced pseudopregnancy or fertile mating.

The observations of thymic weights, thymocyte counts and thymic cell densities yielded few statistically significant differences, but those observed may be biologically important. On day 4 of both pseudopregnancy and syngeneic pregnancy, the thymic weights and thymocyte counts were significantly greater than those on the same day of the oestrous cycle. Furthermore, despite the absence of significant weight changes, the thymocyte counts on days 2 and 3 of pregnancy were significantly greater than those on the comparable days of the oestrous cycle, and on day 2 of pregnancy significantly greater than on the same day of pseudopregnancy. These observations were reflected in the significantly greater thymic cell density on day 2 of pregnancy compared with those of the same day of pseudopregnancy and the oestrous cycle. The significant drop in thymic weight and cell counts on day 4 of oestrous cycle, when compared with the same days of pseudopregnancy and pregnancy, indicate that the hormonal events associated with the interruption of the oestrous cycle have an influence on the thymus and modify the toxic effects of sex steroids on the thymus, while the earlier differences in thymocyte counts and thymic cell densities observed during pregnancy compared with the oestrous cycle and pseudopregnancy indicate that some factor associated with mating has an additional effect upon the thymus. These observations of tissue weight, cell content and cell density include significant differences between the thymic responses to the physiological events characteristic of each state. By extending these observations to include daily levels of thymocyte proliferation, it was anticipated that these differences might be confirmed. During the oestrous cycle, thymocyte proliferation peaked on day 2 and was significantly greater on days 2 and 3 than on any other day of the cycle. Having observed no significant changes in the thymic weight, cell content or cell density during the oestrous cycle, this significant mid-cycle increase in thymocyte proliferation was unexpected. The most feasible explanation of these unchanging thymic parameters in the presence of increased thymocyte proliferation is a comparable increase in the export of T-lymphocytes from the thymus to secondary lymphoid tissues. The mid-cycle increase in thymocyte proliferation is almost certainly caused by ovarian hormones. Unless there is a significant delay between increased hormone secretion and increased thymocyte proliferation, this increased mitotic activity within the thymus is possibly oestrogen-dependent since oestrogen levels begin to increase during early dioestrus while progesterone levels remain very low until midway through prooestrus.[47] It is known that oestrogen stimulates mitotic activity in endometrial epithelium and stroma during the preovulatory phase of the human menstrual cycle.[48] Since there are oestrogen receptors in the thymus,[49] this hormone may indeed be responsible for the increased thymocyte proliferation observed during the oestrous cycle. A repetitive oestrogen-dependent mid-cycle increase in thymocyte proliferation would appear to be a preparation for the coital challenge, which the female rat will allow only during the oestrous phase of the ovarian cycle.

After mechanical induction of pseudopregnancy, thymocyte proliferation was significantly greater on day 3 than on any other day during the observation period. On days 2, 3 and 4, it was significantly greater than on day 5. Since there are no significant changes in thymic weights, thymocyte counts or thymic cell densities during the first five days of pseudopregnancy, it would seem that, as in the case of the oestrous cycle, thymocyte proliferation during early pseudopregnancy is accompanied by an equivalent outflow of T-cells to the secondary lymphoid tissues. This peak of proliferative activity during pseudopregnancy, however, occurred on day 3, some 24 hours later than the peak of proliferative activity during the oestrous cycle. Since thymocyte proliferation and oestrogen secretion both peak on day 3 of pseudopregnancy, it would seem reasonable to conclude that, as during the oestrous cycle, thymocyte proliferation during early pseudopregnancy is oestrogen-dependent.[36] The significantly lower level of thymocyte proliferation on day 5 of pseudopregnancy compared with those on days 2, 3 and 4 suggests that this is a response to increasing levels of progesterone secretion which begin between days 4 and 6 of pseudopregnancy and are sustained until day 9 or 10. Since mechanical induction of

pseudopregnancy does not expose the female reproductive tract to either the ejaculate or the products of conception, the thymic events of early pseudopregnancy are probably controlled solely by the neuroendocrine system.

Following syngeneic mating, thymocyte proliferation was significantly greater on days 3 and 4 than on any other post-coital day. Again the absence of significant changes in the other thymic results during the first 5 days of pregnancy suggests either an equivalent exodus of T-cells to the secondary lymphoid tissues or some immunological mechanism operating within the thymus to achieve clonal deletion during T-cell development.[50] The peak proliferative response during early pregnancy occurred on day 3, as it did during early pseudopregnancy, but following mating this level of proliferation was sustained until day 4 before it fell to the control oestrous phase level on day 5. Since the profiles of ovarian hormone secretion are identical during the 5 days following mechanical induction of pseudopregnancy and syngeneic mating, this altered pattern of thymocyte proliferation in early pregnancy compared with pseudopregnancy suggests that factors other than ovarian hormone secretion, influence the early gestational thymus.

When comparing thymocyte proliferation during the oestrous cycle with that during early pseudopregnancy, there was significantly greater mitotic activity on days 3 and 4 of pseudopregnancy than during the oestrous cycle. Furthermore, during the oestrous phase of the cycle, which in the unstimulated female rat recurs every 5 days, thymocyte proliferation was significantly greater than on day 5 of pseudopregnancy. These significant differences between thymocyte proliferation during the oestrous cycle and early pseudopregnancy confirm the observations already made when considering each of these two physiological states separately. In addition, they may validate the assumption that while thymocyte proliferation is oestrogen-dependent, it can be suppressed by increasing levels of progesterone secretion. Such a validation would certainly require further studies on foetal thymic organ culture where oestrogen-dependency can be elucidated.

Given the assumption that any physiological interruption of the oestrous cycle will produce comparable changes in ovarian hormone secretion, it might be expected that, should thymic proliferation be determined solely or principally by endocrine factors, a comparison of thymocyte proliferation during the oestrous cycle with that during either early pseudopregnancy or early syngeneic pregnancy would present the same pattern of significant differences. While this is true for days 2, 3 and 4, it is not so for day 5 when, contrary to the comparison with early pseudopregnancy, no significant differences between the levels of thymocyte proliferation during the oestrous cycle and early pregnancy were observed on this day. This suggests that thymocyte proliferation during early pregnancy is influenced by other factors apart from ovarian hormones. This was confirmed by comparing thymocyte proliferation during early pseudopregnancy with that during early pregnancy, which demonstrated a significantly greater level of mitotic activity on day 3 of pseudopregnancy and on day 5 of pregnancy.

The absence of significant differences between the daily observations of thymic weights, cell counts and cell densities despite significant increases in thymocyte proliferation during each of the three physiological states suggests either an equivalent exodus of T-cells or some intrathymic mechanism of cell deletion. The significant differences in thymocyte proliferation between the three states suggest a principal role for oestrogen in effecting increased mitotic activity within the thymus. However, the significant increase in both total thymocyte count and thymic cell density on the second post-coital day, the significant depression of thymocyte proliferation on the third post-coital day and the significant increase in thymocyte proliferation on the fifth post-coital day relative to the values recorded on the comparable days of pseudopregnancy, suggest that other factors apart from oestrogen influence the early gestational thymus. The probable cause of the differences observed on days 3, 4 and 5 is the presence of the ejaculate and/or conceptual products in the upper female reproductive tract, since certain components of seminal plasma are known to have a direct immunosuppressive effect. Early mammalian embryos also produce immunosuppressive factors.[51,52] The increased thymocyte proliferation on the fifth post-coital day not only coincides with the onset of implantation but occurs during a period of increasing progesterone secretion. This suggests either a programmed thymic response to ejaculated spermatozoa, initially rendered non-immunogenic by their suspension in seminal plasma, or a response to the process of implantation.

At coitus, large numbers of spermatozoa invade the endometrial glands.[53] While it has been shown that a specific anti-male strain cytolytic T-cell response in the regional lymph nodes continues for 5 days after subcutaneous challenge or artificial insemination of epididymal spermatozoa,[24,30] the comparable post-coital response is limited to the second and third days and is no longer detectable at the time of implantation on day 5.[30] This 'switch-off' of the cytolytic T-cell res-

ponse to mating during the pre-implantation period suggests that some factor in the seminal fluid exerts a suppressive function on the female host's immune response to spermatozoal antigens. A limited cytolytic T-cell response to coitus resulting from the effects of seminal fluid is clearly beneficial in reproduction, but may have adverse effects for the host when exposed to virally infected ejaculate. Since the host's defence against viral infection is predominantly a cell–mediated response, it is particularly relevant, for there is now a well established aetiological link between human papilloma viral infection and cervical neoplasia.[54]

CONCLUSION

It seems reasonable to suggest that proliferative activity in the female thymus is hormonally driven and that the timing of this proliferative activity seems to be programmed to reflect the needs of the immune system in its putative response to mating and implantation. The depression of intrathymic proliferative response consequent to pregnancy is perhaps induced by seminal plasma and may safeguard the implanting conceptus.

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